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Field Sampling of Alfalfa for the Estimation of Guthion Residues[1]

P. A. DAHM,[2] J. GURLAND,[3] E. T. HIBBS,[2] W. H. ORGELL,[2] W. O. PFAEFFLE,[2] and I. LEE,[3] *Iowa State University, Ames*

ABSTRACT

Estimates of Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) residues on alfalfa have been determined by collecting three types of samples and performing chemical analyses and biological assays on extracts of these samples. A 7.6-acre field of alfalfa was sprayed with a water emulsion of Guthion at the rate of 1 pound of Guthion per acre. A two-stage sampling scheme was used to obtain single, composite, and cross-plot composite samples at intervals of 1 day and 3, 7, 14, 21, and 28 days following spraying. For each sampling day, 9 plots, 36 feet by 36 feet, were chosen at random; then 14 subplots, 2 feet by 2 feet, were similarly chosen from within each plot. All the alfalfa within a subplot constituted a sample. The alfalfa in 2 of the subplots in each of the 9 plots was analyzed separately as single samples, the alfalfa from 10 of the remaining 12 subplots was used to form 2 composite samples, each consisting of the combined alfalfa from 5 subplots. The alfalfa in the remaining two subplots in each plot was used to form two cross-plot composite samples. This design provided 228 samples for analysis.

One hundred-gram, well-chopped portions of each alfalfa sample were extracted by a procedure that yielded combined surface and internal residues of Guthion. A decolorized benzene extract was ultimately obtained and aliquots of this solution were used for colorimetric analyses and bioassays. The residue data obtained by both analytical procedures and for the three types of samples were very similar. As an example of these data, the weighted mean chemical residue values of single and composite samples were 51.5, 23.6, 8.7, 1.4, 0.2, and 0.2 p.p.m., respectively, for samples gathered at the six intervals after spraying that are listed above. Because the residues in the last three sets of samples were very low, it was not possible to estimate these residues accurately by biological assays even though a fortifying procedure was used. The residue data indicated that with this sampling scheme and a uniform stand of alfalfa, little, if any, advantage results in using composite samples over single samples to estimate mean residues. For practical purposes, if one were to adopt the two-stage sampling method used in this study for residue estimates involving more than one insecticide and rate of application, a convenient combination would consist of collecting five subplot samples (either single or composite) selected from three plots.

Additional information was obtained concerning the variation in sample weights and moisture, sample processing, and analytical procedures.

Although much research has been done on the determination of insecticide residues on forage crops, insufficient attention has been given to the sampling methods used and to an evaluation of the sampling techniques (Fahey 1954, Gunther & Blinn 1955, Lykken *et al.* 1957, Poos *et al.* 1950, and Wadley 1952). In order to evaluate techniques of sampling alfalfa for residue determinations it seemed desirable to choose an insecticide that could be used potentially to control insects destructive to alfalfa and for which a reliable chemical analytical method was available. These requirements appeared to be met by the organophosphorus insecticide Guthion (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3,-benzotriazine-3-methyl) phosphorodithioate).

In addition to many insect control studies, the action of Guthion in animals and plants has also been investigated. DuBois *et al.* (1957) found that Guthion had a weak inhibitory action, *in vitro*, on the cholinesterase activity of brain, submaxillary gland, and serum of rats. However, intraperitoneal administration of the compound to rats caused marked inhibition of the cholinesterase activity of brain and submaxillary gland, but the activity of serum was unaffected. Murphy & DuBois (1957) have shown that Guthion was enzymatically converted by liver homogenates to an agent with strong anticholinesterase properties; however, this metabolite lost its activity rapidly through enzymatic hydrolysis. The enzyme system which oxidized Guthion to an anticholinesterase agent was localized intracellularly in the microsomes. March *et al.* (1957) proposed that the metabolism of Guthion by the white mouse and the American cockroach, *Periplaneta americana* (L.), involves oxidation of the thiono sulfur (P=S) of Guthion to form the thiol (P=O) analogue, which is an extremely effective inhibitor of cholinesterase. Subsequent hydrolysis of this metabolite produced compounds of low toxicity. In contrast to these animal metabolism studies, Tietz *et al.* (1957) apparently did not find any of the thiol analogue of Guthion

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in leaves of cotton plants sprayed with the P^{32}-labeled insecticide.

In normal agricultural practice a field of alfalfa is generally sprayed with a single insecticide at an intended single rate of application. To measure the residues of this insecticide, samples of alfalfa are usually gathered at specified time intervals following spraying to obtain estimates of the true mean amount of residue present in the field at such times. Various types of composite alfalfa samples have been used to estimate insecticide residues on alfalfa without determining whether these composite samples actually provide estimates that are more reliable than those based upon single samples. Therefore, the sampling plan used in this study was designed to compare single and composite methods of sampling for estimating mean residues. A second objective was to compare the estimates of insecticide residues obtained by chemical analyses and biological assays.

MATERIALS AND METHODS.—*Sampling Design.*—Approximately 7.6 acres of alfalfa were selected from a 140-acre field of unsprayed, second cutting, second year alfalfa, *Medicago sativa* L., var. Ranger, located on the Iowa State University farm near Ankeny, Iowa. This square area was divided into 196 plots, 36 feet by 36 feet; thus there were 14 rows of plots, each containing 14 plots (fig. 1). A swath was mowed between plots and around the margins of all the plots. These marginal alleys were kept mowed throughout the experiment. The plot size was chosen to provide 324 subplots, 2 feet by 2 feet.

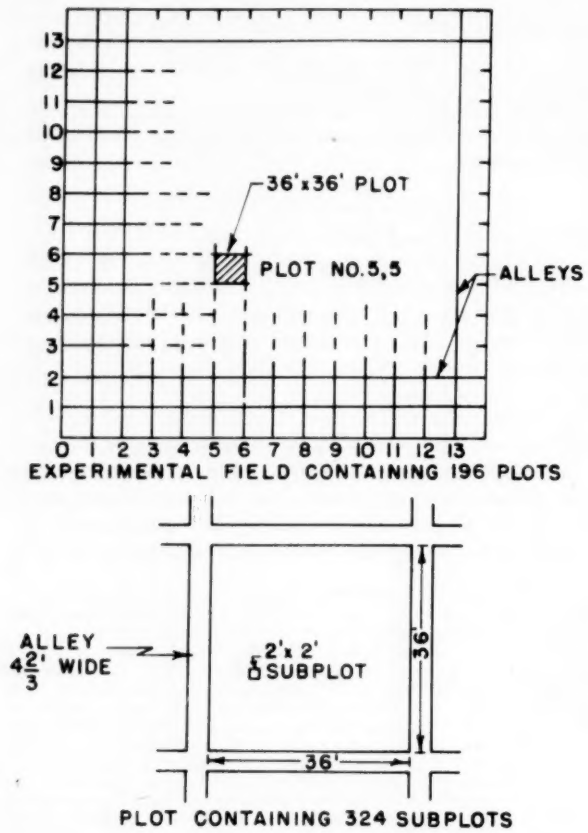

FIG. 1.—Experimental design of alfalfa plots used for sampling studies of Guthion residue dissipation.

A two-stage sampling scheme was used to obtain samples based on an unbiased scheme of randomization. Samples were collected 1 day and 3, 7, 14, 21, and 28 days following the insecticide application. For each sampling day, nine plots were chosen at random, using tables of random numbers. Then 14 subplots within each of the 9 selected plots were similarly chosen. The subplots were located by pacing along the edges of each plot, allowing 3 feet per step. The boundaries of each subplot were marked with an angle iron quadrat, 2 feet by 2 feet square. All the foliage within each subplot was cut approximately 1 inch above the ground. The alfalfa was separated from the weeds and placed in polyethylene bags. A two-stage sample, involving the random selection of plots and subplots, was taken because of the intuitive expectation that the variation among plots would be greater than the variation among subplots within plots. For although the stand of alfalfa appeared to be fairly uniform, one would expect two subplots widely separated in the 7.6-acre area to show greater difference, on the average, from one another than two subplots in the same plot. The data collected on the basis of this sampling scheme afforded an opportunity to test the relevance of such a theory.

Single Samples.—For each sampling day, the alfalfa in two of the subplots selected in each of the nine plots was collected and analyzed separately as two "single samples." The arithmetic means of chemical analyses and biological assays of extracts prepared from aliquots of these 18 samples provided "single sample estimates" of the true mean residue of the plot area. The two residue values from each of the nine plots afforded a means of estimating the component of variance ascribable to "within plots" variation, while the nine mean values based on the arithmetic mean of the two residue values from each plot afforded the possibility of estimating the component of variance ascribable to "between plots" variation. These components enabled the variance of the single sample estimate of the mean residue value to be computed.

Composite Samples.—The alfalfa from 10 of the remaining 12 subplots in each plot was used to form two "composite samples," each consisting of the combined alfalfa from five subplots. The arithmetic means of chemical analyses and biological assays of extracts prepared from aliquots of these 18 composite samples provided "composite sample estimates" of the true mean residue of the plot area. Just as in the case of the single sample estimates, it was possible to estimate the components of variance ascribable to "between" and "within" plot variation using the residue data from the composite samples. A comparison was also made between the variances of the single and composite sample estimates. The residue data from the single and composite samples also provided an estimate of what has been designated as a "weighted mean residue of single and composite samples." The latter residue values were obtained by weighting the single and composite sample estimates inversely as their variances.

Cross-plot Composite Samples.—The alfalfa in the remaining two subplots in each plot was used to form "cross-plot composite samples." Each of these samples

consisted of the combined alfalfa in one of the two subplots remaining in each of the nine plots selected for the particular sampling day. As there were only two "cross-plot composite sample estimates," it was not possible to estimate reliably the variance of the arithmetic mean of these two sample estimates. However, it was possible to compare these estimates of the mean residue with those based on single and composite samples.

To complete the sampling schedule described above it was necessary to locate and gather alfalfa from 126 subplots per sampling day (18 for the single samples, 90 for the composite samples, and 18 for the cross-plot composite samples). In some instances when gathering the 1-, 3-, and 7-day samples, a subplot chosen for a single sample contained less alfalfa than the 100 gm. needed for analysis purposes. In this case, the alfalfa from this subplot was included as one of the five subplots in a composite sample for that plot, and a different subplot, containing sufficient alfalfa, was selected at random for the single sample. In addition to these samples from the sprayed area, approximately 15 subplot samples of untreated alfalfa were collected each sampling day from the unsprayed portion of the alfalfa field for use as control samples in the chemical analyses and biological assays.

Insecticide Application.—The experimental area, including alleys and margins, was sprayed on July 16, 1957, with a water emulsion of Guthion at the rate of approximately 1 pound of Guthion per acre. An emulsifiable concentrate formulation of Guthion, containing 1.5 pounds of Guthion per gallon,[4] was used to prepare a water emulsion which contained one pound of Guthion per 10 gallons of spray. The spray was applied at the rate of 10 gallons per acre with a Jeep-mounted sprayer[5] equipped with a 24-foot spray boom fitted with 15 flat, fan-type nozzles that provided an effective spray width of 25 feet. The nozzles were about 20 inches above the tops of the alfalfa plants during spraying. The alfalfa was about 1½ feet high at the time of spraying.

Sample Processing.—The alfalfa samples were transferred from the field to a refrigerator box for cooling. They were soon removed from the box, weighed, chopped into approximately 2-inch sections using a feed cutter,[6] and, except for small amounts which were withheld for determinations of the water content, were stored at about −15° C. About 5 hours were required to complete the procedures from field sampling through cold storage. Within a few days, or a few weeks at most, the samples were removed from cold storage, chopped further, and thoroughly mixed in a commercial food chopper.[7]

Five samples were prepared for the determination of their water content each time alfalfa samples were collected for residue determinations. Three of the five samples were formed by combining small amounts of alfalfa from the nine composite samples (three composites per water content sample) and the other two samples were obtained from each of the cross-plot composite samples. These alfalfa samples were placed in cloth bags and dried for 1 week at about 60° C., after which the water content of the samples was calculated. The weights of the single and composite samples were used to compute the variances within plots as follows: (1) the total sums of squares were obtained from the individual sample weights, (2) the sums of squares for "between plots" were computed, and (3) the variances "within plots" were obtained by subtracting the values obtained in step (2) from the values obtained in step (1) and dividing this quantity by nine degrees of freedom.

The most exacting aspects of estimating insecticide residues usually are the cleanup treatments to which the samples must be subjected before analysis can be accomplished. Our goal was to prepare a solution that could be used for both chemical analyses and biological assays. The extraction procedure used was designed to yield combined surface and internal residues of the plant sample (MacDougall 1957). The solvents (C. P. grade) were distilled in an all-glass apparatus before being used. This precaution is especially necessary with benzene because various grades of this solvent contain an impurity which gives a magenta color with spectral characteristics identical to those obtained with parathion, using the Averell & Norris (1948) colorimetric method (Edwards 1949). A 100-gram, well-mixed subsample of twice-chopped alfalfa was placed in a Waring blendor jar and 150 ml. of distilled water added. A thin sheet of rubber was placed over the top of the blendor jar and the jar cover pressed into place to prevent the material being blended from leaking out of the jar. The material was blended at high speed for 2 minutes to produce a homogeneous mixture; 250 ml. of acetone were added and the contents blended for an additional 3 minutes. The slurry thus obtained was transferred quantitatively to a 1-quart, glass Mason jar. The blendor jar was washed three times with approximately 15 ml. of acetone each time and the washings added to the Mason jar. A mechanical shaker was then employed to shake the slurry for an hour.

The slurry was suction-filtered through a Büchner funnel. The quart jar was rinsed several times with portions of a 1:1 mixture of acetone and water (total volume of rinses about 100 ml.); these rinses were poured over the filter cake in the funnel to elute any Guthion residues remaining in the filter cake. The filter cake was allowed to suck dry. The green acetone-water filtrate was transferred quantitively to a 1,000-ml. Squibb separatory funnel and extracted twice with 50-ml. and four times with 25-ml. portions of chloroform. To remove any water trapped in the chloroform, the combined extracts were filtered through a 1-inch layer of anhydrous sodium sulfate held in a cylindrical glass funnel plugged with glass wool. The sodium sulfate was washed twice with 25-ml. portions of chloroform. The chloroform extract and washes were collected in a 600-ml. beaker and evaporated to a green, sirupy residue on a hot water bath, using a gentle current of air to facilitate evaporation.

The dark green residue remaining in the beaker was dissolved in 25 ml. of benzene and passed with the aid of suction through 10 grams of a mixture consisting of three parts, by weight, of Attapulgus clay[8] (adsorbent) and one part Hyflo Super-Cel[9] (filter aid) in a 125-ml. Squibb separatory funnel. The beaker containing the residue

[4] Obtained from the Chemagro Corp., Kansas City, Missouri.

[5] Designed and constructed by E. T. Hibbs, Department of Zoology and Entomology, Iowa State University, and W. G. Lovely, Agricultural Research Service, U. S. Department of Agriculture (stationed at Iowa State University).

[6] Ohio Feed Cutter, Model 8½, K-2, The Silver Mfg. Co., Salem, Ohio.

[7] Hobart Food Chopper, Model 84141, Hobart Mfg. Co., Troy Ohio

[8] Obtained from Minerals & Chemicals Corp. of America, Menlo Park, New Jersey.

[9] Obtained from Johns-Manville Products Corp., New York, New York.

was rinsed twice with 50-ml. portions and twice with 25-ml. portions of benzene. Finally, the adsorbent mixture was washed with three additional 25-ml. portions of benzene. The combined benzene elutriates were transferred from the filter flask to a 250-ml. graduated cylinder and made up to a volume of 250 ml. with small portions of benzene which were used to rinse the filter flask. This solution was then transferred to a 1-pint, glass bottle; the latter was labeled and stored in the dark until the contents were used for chemical analyses and biological assays. If the benzene solution was not water clear after passing through the adsorbent mixture, it was rejected and another alfalfa subsample was carried through the procedure. This requirement was especially necessary for the biological assay procedure in order to avoid excessive masking effects as described below. A series of recovery experiments was run concurrently with each set of field samples. The amounts of Guthion (in acetone) added to 100-gm. samples of untreated, control alfalfa corresponded roughly to the range of Guthion residues in the field samples.

In order to learn more about the quantitative fate of Guthion residues carried through these sample processing procedures and to discover ways of reducing the loss of Guthion residues, the following processes were studied in some detail: (a) efficiency of the chloroform extraction of Guthion from the acetone-water mixture, (b) effect of time and temperature relationships on the loss of Guthion during evaporation of the chloroform-acetone extract, and (c) adsorption of Guthion, the thiol (P=O) analogue of Guthion, and interfering substances by various decolorizing mixtures. Acetone-water mixtures, approximating the final ratio of these two liquids in the extraction process, containing 1,000 μg. of Guthion were extracted successively with six portions of chloroform as described above. The Guthion added was 99% recovered. The mean recoveries of Guthion from the first and second chloroform extracts were 88.4 and 7.6%, respectively. These figures suggested that very little Guthion would be lost during the chloroform extraction process. A study of time and temperature effects on the loss of Guthion during evaporation of the chloroform-acetone extract showed that the most efficient evaporation occurred when the water bath was maintained between 60° and 70° C. Several adsorbent substances were combined with Hyflo Super-Cel to evaluate their effectiveness in removing plant pigments without adsorbing Guthion excessively. Combinations of Attapulgus clay and Hyflo Super-Cel (*e.g.* in ratios of 1:3, 1:1, and 3:1) were superior to all others in decolorizing the solution and allowing most of the Guthion to pass through the adsorbent. A 3:1 ratio of Attapulgus clay to Hyflo Super-Cel was required to decolorize the dark green solutions obtained with alfalfa. From 10 to 20% of the Guthion was retained during the adsorption process. Retention of Guthion in the adsorbent column was roughly proportional to the amount of Attapulgus clay in the adsorbent mixture. Essentially none of the Guthion was retained by the quantities of Hyflo Super-Cel used in these mixtures. Although mixtures of Darco G-60 with either Hyflo Super-Cel or Celite 545 allowed rapid filtrations, they adsorbed slightly more Guthion than corresponding Attapulgus clay and Hyflo Super-Cel mixtures. The thiol analogue of Guthion used in these experiments (see below) was adsorbed completely by the 3:1 Attapulgus clay and Hyflo Super-Cel mixture.

Colorimetric Procedure.—Three methods have been reported for the colorimetric estimation of Guthion (Giang & Schechter 1958, MacDougall 1957, and Wollenberg & Schrader 1956). The method reported by MacDougall (1957), which was used in this study, is an adaptation of the Bratton & Marshall (1939) reaction involving the coupling of certain diazotized compounds with *N*-(1-naphthyl) ethylenediamine dihydrochloride in acid solution to form a magenta-colored complex. This method was used by Averell & Norris (1948) to estimate small amounts of parathion, after reduction to the corresponding amino compound.

Small amounts of Guthion in isopropanol can be hydrolyzed with an aqueous solution of potassium hydroxide to yield *o*-aminobenzoic acid (anthranilic acid). The latter compound is diazotized and coupled with *N*-(1-naphthyl) ethylenediamine dihydrochloride to form a magenta-colored solution. The details of this method have been thoroughly described by MacDougall (1957) and will not be repeated here. The magenta-colored solutions were read 90 minutes after the addition of the coupling reagent; the absorbancies of the solutions were determined with a Beckman, Model DU, spectrophotometer, using a wave length setting of 555 mμ, a slit width of 0.065 mm., and glass cells with a 1-cm. light path. A standard curve, plotting absorbance values against micrograms of Guthion, was prepared with each set of analyses. The quantities of Guthion found by this method were converted to parts per million of Guthion based on the freshly harvested weight of the sample. The 95% confidence intervals for the residue values obtained by chemical analyses were computed for 18 single and 18 composite residue values for each day of sampling. The variances within plots of the residue values obtained by chemical analyses were computed in the same manner as the "within plot" variances of the sample weights.

A Guthion analytical standard sample[4] (m.p. 71–72° C., No. F7107) was used for both the chemical analyses and biological assays. A sample of the thiol (P=O) analogue of Guthion,[4] *O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorothiolate (m.p. 76.5–77.5° C., Lot No. 1) was used for comparative colorimetric analyses. The infrared spectra of these samples, using carbon tetrachloride and carbon disulfide separately as solvents, indicated significant differences as follows: broad absorbance bands at approximately 650 and 820 cm.$^{-1}$ wave numbers were due to P=S stretching vibrations in the case of the Guthion sample (CS_2 as solvent) and a narrower absorbance band indicated a P=O stretching vibration at about 1,250 cm.$^{-1}$ for the thiol analogue (CCl_4 as solvent). A weak but well-defined absorbance band occurred for both samples at about 1,180 cm.$^{-1}$ indicating the presence of P-O-CH_3 groups. The ultraviolet spectra of both samples (CCl_4 as solvent) showed a primary absorbance peak at 303 mμ. and a second peak at 316 mμ. Another well-defined peak was observed at 292 mμ. in the case of the thiol analogue.

Biological Assays.—An indirect method of bioassay was employed in which the mortality of the test insect following exposure to a fixed dose of the insecticide was

measured (Bliss 1956). Each standard and test assay consisted of three dose levels so chosen that the resulting mortalities of the test insects were expected to fall between 15 and 85%. If the test preparation is truly a dilution or concentration of the standard, the two dose-response regression lines must be parallel, assuming that the relationship of response versus log-dose is linear (Burn *et al.* 1950). A laboratory culture of house flies, *Musca domestica* L., was used to provide the 4- to 5-day old female flies used in the bioassays. Fifty flies were used per dose in both test and standard assays. All the flies used for one assay series were approximately the same age and were taken from the same holding cage. The flies were reared according to the procedure specified by the Chemical Specialties Manufacturers Association for the Peet-Grady method of testing fly sprays (Anonymous 1958). The test chambers consisted of 1-quart, wide-mouth, glass Mason jars fitted with a screw cap and wire screen to confine the flies.

A measured volume of either an analytical standard solution of Guthion in benzene (standard preparation) or an aliquot of the plant extract solution prepared as described above (test preparation) was added to each jar. In order to conserve parallelism between the standard and the test regression lines, it was necessary to have the same masking effect owing to the plant extract in both the standard and the test preparations. This was done by adding the same total amount of alfalfa extract (test or control extracts by themselves or a combination of these two extracts) to all three dose levels of the test assays and the corresponding levels of the standard assay. A typical standard assay series (in this case, quantities used to evaluate residues in samples gathered 7 days after spraying) consisted of the following quantities of Guthion and insecticide-free, control alfalfa extract: 25, 30, and 40 μg. of Guthion plus 10 ml. of control extract for each dose. The corresponding test assay series consisted of 5, 7.5, and 10 ml. of the test extract plus 5, 2.5, and 0 ml. of control extract, respectively. The insecticide residues were so low in the extracts prepared from samples collected 14, 21, and 28 days after spraying that the flies did not respond consistently to the assays. An attempt was made to estimate these residues by fortifying the test extracts with known amounts of the standard solution. Whenever the test extracts were so fortified, the control extracts (used to provide the same masking effects in standard and test assays as illustrated above) were similarly diluted with benzene in order to conserve parallelism between the standard and the test regression lines.

Preparation of the bioassay jars was completed by adding 0.2 mg. of corn oil (Mazola) to each jar. The jars were then rolled mechanically in a horizontal position to evaporate the solvent and distribute the toxicant on the inner walls of the jars. Fifty female flies were placed in each jar. The treated jars were placed horizontally on a shelf for 24 hours at 80 ± 2° F. after which the number of dead and moribund flies were counted. The flies were fed a mixture of sugar and diluted condensed milk during the exposure period. The number of moribund flies was included with the dead flies in the statistical analyses of the bioassay data. When necessary, a correction was made for mortality among the control flies (Abbott 1925).

The relative potency (R) of each test preparation was estimated by the minimum logit chi-square method (Berkson 1953). This method was used because the estimates were easily determined and the data appeared to be closely fitted by the logit transformation. The relative potencies were converted to parts per million (p.p.m.), based on the freshly harvested weight of the samples, by multiplying the relative potency (μg./ml.) by the volume (250 ml.) of the final extract and dividing this product by the weight (100 gm.) of the alfalfa sub-sample. Thus the number of p.p.m. equals 2.5R.

Computing Confidence Intervals.—The 95% confidence interval for the residue values obtained by chemical analyses and biological assays was computed for 18 single and 18 composite residue values for each day of sampling. To calculate the weighted mean residue values of single and composite samples ($\bar{R}$) for each sampling day, the reciprocals of the variances of the mean residue values of the single and composite samples were used as weighting factors in the following formula (cf. Burn *et al.* 1950):

$$\bar{R} = \frac{\bar{R}_s/V(\bar{R}_s) + \bar{R}_c/V(\bar{R}_c)}{1/V(\bar{R}_s) + 1/V(\bar{R}_c)}$$

where:

$\bar{R}_s$ = mean residue values of single samples
$V(\bar{R}_s)$ = variance of mean residue values of single samples
$\bar{R}_s$ = mean residue values of composite samples
$V(\bar{R}_s)$ = variance of mean residue values of composite samples.

The variances within plots of the residue values obtained by chemical analyses and biological assays were computed in the same manner as the "within plot" variances of the sample weights. This procedure has been described above. The 95% confidence intervals for the weighted mean residue values of single and composite samples were obtained by using the estimated variances, $V(\bar{R})$, as computed from the following formula (cf. Burn *et al.* 1950):

$$V(\bar{R}) = \frac{V(\bar{R}_s)\ V(\bar{R}_c)}{V(\bar{R}_s) + V(\bar{R}_c)}$$

Weather Conditions.—A good estimate of the temperatures and precipitation prevailing during these experiments was available from the records of the Ankeny Weather Bureau station (U. S. Dept. Commerce 1957) located about 1 mile from the plots. A brief summary of this information for the 4-week period after spraying follows: the over-all mean temperature during the period was 77.3° F.; the mean temperatures for the four weekly periods after spraying were 80.9°, 76.4°, 73.1°, and 78.5° F., respectively. A total of 1.39 inches of rain fell during the 28-day period; this precipitation was distributed as follows: 0.42, 0.10, 0.65, 0.10, and 0.12 inches on the 5th, 11th, 12th, 14th, and 28th day, respectively, after spraying.

RESULTS AND DISCUSSION.—*Residue Data.*—A summary of the residues of Guthion as obtained in these experiments is presented in table 1. The weighted mean chemical residue values of single and composite alfalfa samples are portrayed in figure 2, using a semilogarithmic plot. The residue data obtained by both chemical and biological procedures are very similar for the first three

Table 1.—Residues of Guthion, in parts per million, obtained from chemical analyses and biological assays of alfalfa samples gathered from a 7.6-acre field sprayed with a water emulsion of Guthion at the rate of 1.0 lb. of Guthion per acre.[a]

Days After Spraying	Single Samples[b]		Composite Samples[c]		Cross-Plot Composite Samples[d]	
	Chemical Analyses	Bioassays	Chemical Analyses	Bioassays	Chemical Analyses	Bioassays
1	51.6±2.5	54.5±4.6	51.3±2.7	56.1±5.6	54.8	47.2
3	23.5±1.8	23.5±2.5	24.1±3.1	26.1±1.8	25.7	28.2
7	8.1±1.0	10.0±1.1	9.1±0.9	10.7±1.3	10.1	10.0
14	1.4±0.4	—[e]	1.4±0.3	—[e]	1.4	—[e]
21	0.2±0.1	—[e]	0.2±0.1	—[e]	0	—[e]
28	0.1±0.1	—[e]	0.3±0.2	—[e]	0	—[e]

[a] All values are based on the freshly harvested weight of the samples.
[b] 95% confidence intervals, based on 18 single samples per sampling day.
[c] 95% confidence intervals, based on 18 composite samples per sampling day, each composite sample consisting of five single samples.
[d] These are the mean values of two composite samples per sampling day, each composite sample consisting of nine single samples.
[e] Because of the low residues in these samples, it was not possible to obtain an accurate estimate of the residues by the fortified bioassay procedures used.

sets of samples, collected 1, 3 and 7 days after spraying. The residues in the last three sets of samples, collected 14, 21, and 28 days after spraying, were too low to estimate by the same bioassay procedure employed for the first three sets of samples. Extracts of these last three sets of samples were fortified with known amounts of insecticide and aliquots of these solutions assayed against standard preparations as described above. The residues computed from the relative potency values obtained by these assay procedures were not consistent with the toxicity to flies of the unfortified extracts. Further work is contemplated to determine whether low residues, like those encountered in the last three sets of samples in these experiments, can be estimated accurately by fortifying procedures. It was also difficult to estimate precisely by chemical analysis the low residues (*e.g.* about 0.2 p.p.m.) in the 21- and 28-day samples. Because of the low residues, it was necessary to use large aliquots of the benzene extracts for the colorimetric analyses. Although no color was apparent when these aliquots were evaporated to dryness, the ensuing isopropanol solutions were yellowish in color and when the coupling reagent was added, the solutions became more nearly salmon- than magenta-colored.

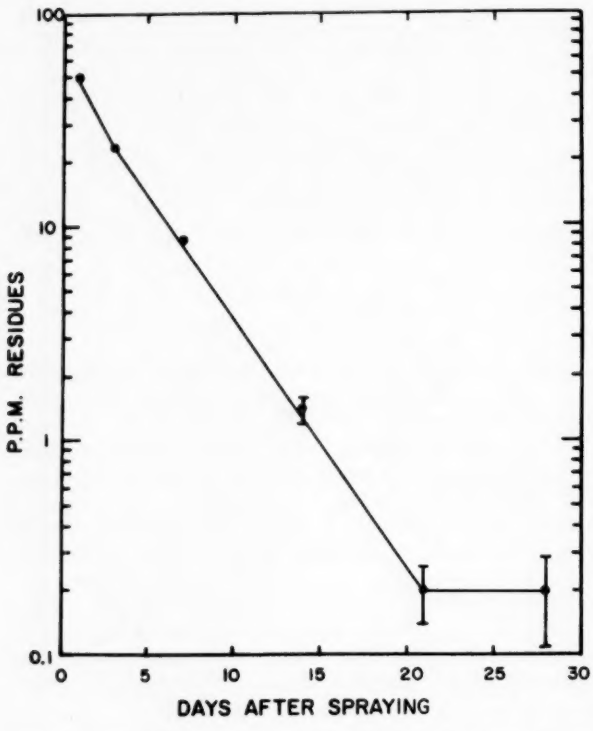

FIG. 2.—The weighted mean chemical residue values of single and composite alfalfa samples gathered from a 7.6-acre field sprayed with Guthion at the rate of 1.0 lb. per acre. The approximate 95% confidence intervals are shown for samples gathered 14, 21, and 28 days after spraying. The confidence intervals for the residue values for the 1-, 3-, and 7-day samples were too short to be discernible.

Chemical analysis of an extract prepared from a composite alfalfa sample gathered approximately 1 hour after spraying indicated a Guthion residue of 69.4 p.p.m.; extrapolation of the residue curve in figure 2 back to time zero indicates an initial Guthion residue approximately equal to 70 p.p.m. The estimated half-life of these Guthion residues is 2 days, compared with 2, 5, and 6 days for parathion, lindane, and DDT, respectively (values estimated by Gunther & Blinn 1955, from data of Hopkins *et al.* 1952). These residue data for Guthion are very similar to those reported by Dogger & Bowery (1958) for malathion. Both insecticides were applied at the rate of 1 pound per acre. For malathion, the residues were approxomately 47, 8, 0.8, 0.4, and less than 0.2 p.p.m. on alfalfa samples gathered 1 day, and 5, 10, 15, and 20 and 30 days, respectively, after application (the half-life of malathion residues, estimated graphically from these data, is also about 2 days).

The recovery of Guthion added to 100-gm. samples of untreated, control alfalfa is summarized in table 2. About 8% less Guthion was measured by biological assays of the final benzene extracts than by chemical analyses of the same extracts. A greater range of variation (27%) occurred among the recoveries by biological assay than among the corresponding recoveries by chemical analysis (12%). The average water content of all sets of alfalfa samples was about 72% (table 3). When these rather similar percentages are used to replot the residue values shown in figure 2 on a dry weight basis, the only effect is to shift the curve upward.

Table 2.—Recovery of known amounts of Guthion (in acetone) added to 100-gram samples of untreated, control alfalfa.

Guthion Added		Per Cent Guthion Recovered	
(μg.)	(p.p.m.)	Chemical Analyses	Bioassays
5,000	50	75.5 (4)[a]	88.1 (4)
2,500	25	82.5 (8)	70.2 (4)
1,200	12	86.6 (4)	61.3 (4)
300	3	87.3 (4)	81.6 (4)
Over-all mean recoveries		83.0 (20)	75.3 (16)

[a] The numbers in parentheses indicate the number of analyses or bioassays.

These experiments apparently have not indicated whether any of the Guthion applied to the alfalfa plants was converted to the thiol (P=O) analogue. It was discovered, after the alfalfa samples had been processed, that a compound designated as the thiol analogue of Guthion (described above under colorimetric procedure) was completely adsorbed by the Attapulgus clay used to decolorize the alfalfa extracts. A further comparison of Guthion with this thiol analogue sample showed that both chemicals produced magenta-colored solutions (maximum absorption 555 mμ.) by the colorimetric procedures used in these experiments (MacDougall 1957), the Guthion color having about 1.6 times more absorbancy at 555 mμ. than the color produced by an equal concentration of the thiol analogue. Conversely, the thiol analogue was about 1.5 times more toxic to house flies than Guthion, using the bioassay procedures described above. After comparable exposures to ultraviolet light, Guthion residues responded less strongly to the colorimetric test than residues of the thiol analogue (cf. Frawley *et al.* 1958). It is hoped that some of these findings can be investigated further to enhance our knowledge of the fate of insecticides on plants.

Sampling Procedures.—The sampling procedure used in these experiments and the concomitant large number of samples for residue analysis preclude general adoption of these practices in most residue determination programs. Nevertheless, one can reasonably ask: "What theoretical and practical information can be derived from such an experiment?" It is evident from table 1 that the 95% confidence intervals for the means based on single samples are about the same magnitude as the corresponding intervals for means based on composite sample weights. Furthermore, the within-plot variances for single and composite sample residues obtained by chemical analysis were roughly similar (table 4). Theoretically, one would expect the variance within plots of single sample residue values to be about five times the comparable values for the composite values. Although some restriction to randomness resulted from the selection of additional subplots for single samples when those chosen originally contained too little alfalfa, it is very unlikely this would decrease the within plot variance of single samples to such an extent. These observations imply that in obtaining estimates of mean residues on a crop like alfalfa from the sampling scheme adopted, no advantage is gained in using composite samples consisting of five subplots over the use of single samples. For example, on the basis of the data collected, if one assumes that an estimate of the true mean residue in the field is reliable if its coefficient of variation does not exceed 10% a convenient combination would consist of five subplot samples (either single or composite) selected from three plots.

The growth of plants along with weather effects and leaf drop are important factors in "reducing" insecticide residues calculated on a weight basis. In these experiments the changing pattern of sample weights was curvilinear with the highest weights being observed in the 14-day samples and the lowest weights at the beginning and end of sample collection (table 3). The decrease in weights of the 21- and 28-day samples was not due primarily to a loss of moisture. A possible explanation is found in the large numbers of leafhoppers that were present in the field during the last 2 weeks of the experiment. The variance within plots of single sample weights was expected to be about five times that of the composite samples because each composite sample consisted of alfalfa from five subplots. The actual variance within plots of single sample weights was roughly one to seven times that of the composite samples. On the other hand, the 95% confidence intervals for the means based on single sample weights are practically all shorter than the corresponding intervals for means based on composite sample weights (table 3). This is due to the fact that the

Table 3.—Subplot sample weights and water content of the alfalfa samples.

Days After Spraying	Mean Weights of Subplot Samples			Water Content of Samples[d]
	Single Samples[a] (gm.)	Composite Samples[b] (gm.)	Cross-plot Composite Samples[c] (gm.)	(%)
1	175±23	138±38	183	72.2±1.3
3	192±31	150±43	196	72.2±2.5
7	247±38	178±53	218	72.4±1.0
14	267±43	238±42	293	74.8±0.8
21	257±24	225±48	246	72.1±0.5
28	191±37	139±42	185	67.0±1.9

[a] 95% confidence intervals, based on 18 single samples per sampling day.
[b] 95% confidence intervals, based on 18 composite samples per sampling day, each composite sample consisting of five single samples. These values are one-fifth of the observed weights of the composite samples per sampling day and are so presented for comparison with the single samples.
[c] These values are one-ninth of the mean of two cross-plot composite samples per sampling day and are so presented for comparison with the single samples. Each cross-plot composite sample consisted of nine single samples from the nine selected plots.
[d] 95% confidence intervals, based on five composite samples per sampling day.

Table 4.—Variances within plots of sample weights and residues.

Days After Spraying	Single Samples			Composite Samples		
		Residues			Residues	
	Sample Weights	Chemical Analyses	Bioassays	Sample Weights	Chemical Analyses	Bioassays
1	1,848	92.2	79.5	595	21.9	73.7
3	2,480	13.1	10.1	705	23.6	10.6
7	1,165	3.8	5.1	1,422	3.2	8.0
14	5,492	0.2	—[a]	1,192	0.4	—[a]
21	2,968	0	—[a]	430	0	—[a]
28	1,259	0	—[a]	648	0	—[a]

[a] Because of the low residues in these samples, it was not possible to obtain an accurate estimate of the residues by the fortified bioassay procedures used.

component of variance for "between plots" is larger in the case of composite samples. This fact would suggest that to increase the precision of estimates of composite sample mean weights it would be advisable to use more plots, whereas in the case of single sample estimates it would be advisable to use more subplots.

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Evaluation of Candidate Insecticides and Insect Pathogens for Tobacco Hornworm Control, 1956–1958[1,2]

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ABSTRACT

Three years of laboratory and field testing of materials being screened for control of tobacco hornworms (*Protoparce sexta* (Johan.)) with reduced insecticide residue are reported. Comparative contact toxicity tests indicated the following descending order of effectiveness: Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) = DDVP > endrin = Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) = Shell SD 4402 (1,3,4,5,6,7,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) > Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) = Sevin® (1-naphthyl-*N*-methyl carbamate) > Chlorthion® (*O*,(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate), Delnav® (2,3,-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), ethion, and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate). In field tests Thiodan, Sevin, SD 4402, and Dipterex gave larval control

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generally comparable to endrin at the concentrations tested. Guthion gave poorer results than endrin at comparable concentrations but was effective at the higher dosages. All materials were about equal in reducing hornworm damage when estimations were made 9 days after treatment, but Guthion and Dipterex permitted more damage than the other materials 22 days after treatment. The residues from the compounds dissipated to the extent of 85 % or more during flue-curing. None of the materials affected taste of cured tobacco except Sevin, which caused a slight off-flavor. *Bacillus thuringiensis* Berliner was found to be equal to endrin in preventing hornworm damage although not so effective initially in reducing larval populations. The *Bacillus* organism had no detrimental effect on colonies of *Polistes exclamans* Vier. Two commercial preparations of *Bacillus* were found to be equal to insecticides in preventing hornworm damage.

Tobacco is a crop that can well justify the expense of extensive insect control programs by the grower but can also be drastically affected by materials which cause off-flavors. The expense of producing, curing, and aging tobacco forces an extremely conservative attitude for pesticide recommendations. Even residues that do not affect flavor are objectionable to the manufacturer, who desires a leaf as nearly natural as is available. For these reasons a comprehensive search for less residual materials for control of tobacco insects has continued for the past several years. In addition to the obvious disappearance by weathering, insecticides applied to tobacco may also dissipate during the relatively high temperatures of flue curing. Therefore, an examination of the residual phosphates seemed worthy of investigation.

The results of researches by Allen *et al.* (1953), Rabb & Guthrie (1956), and Dominick (1956) showed that endrin is a valuable insecticide for tobacco insect control. Rabb *et al.* (1957) reported results with *Bacillus thuringiensis* Berliner for tobacco hornworm (*Protoparce sexta* (Johan.)) control. Although the use of Sevin® (1-naphthyl-*N*-methyl carbamate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) have been reported by numerous authors on other crops, nothing has been published on the effect of these recently released insecticides on tobacco. Shell SD 4402 (1,3,4,5,6,7,8-octachloro—3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) has not been reported in field tests.

This paper reports the laboratory-, field-, and taste-test information that has been accumulated for control of the tobacco hornworm, since 1956. Where specific colorimetric methods were available the effect of weathering and barn-curing of tobacco on residues is also indicated.

METHODS AND MATERIALS.—*Laboratory Tests.*—The comparative contact toxicity of the candidate compounds was ascertained before the materials were tested in the field. These tests were made during 2 different years, and endrin was included in each year's tests for standard comparison. The materials were dissolved in dioxane and applied topically using a micrometer-actuated 0.25-ml. tuberculin syringe which delivered the measured amount of the toxicant to the dorsum of the first thoracic segment of 5th instar (4 to 6 grams) field-collected tobacco hornworms. Twenty to forty larvae were used to establish each point on the eye-fitted dosage-mortality curve, and 4 or more points were used to establish the LD-50 figures. Check larvae were subjected to a quantity of dioxane equal to the highest volume of dioxane delivered with the less soluble materials and handled in identical fashion as the treated larvae. After treatment the larvae were provided with green tobacco leaves and held in 1-pint ice cream cartons for 4 days.

Field Tests.—The general procedure was similar for tests of insecticides and pathogens. Guard rows were from two to three rows wide when separating spray-treatment plots and from four to five rows wide when separating dust treatments. Unless otherwise indicated all plots were replicated four times in a randomized block design. All dust treatments were applied with a Hudson rotary hand duster to each side of the row. The full coverage spray treatments were applied with a Hahn high-clearance sprayer operating at 60 p.s.i. and delivering 40 gallons per acre or a compressed air, hand sprayer delivering 50 gallons per acre. All tests were made against second-brood hornworms on tobacco near the topping stage of growth. The number of 3rd, 4th, and 5th instar hornworms was recorded on marked plants selected at random throughout each plot. If infestations were too light or too variable, larvae were carefully moved to the marked plants from adjacent plants. Damage estimates were made utilizing the method described by Lawson & Rabb (unpublished manuscript).

Insecticides.—In 1956, five dust formulations were compared on single-row plots, 55 feet long. The number of living larvae on 20 marked plants and the number of dead larvae on the plants or in the row middles were recorded at the indicated intervals. In 1957, four spray formulations (high-clearance sprayer) and one dust formulation were tested on two-row plots, 40 feet long. One and one-half inches of rain occurred 3 hours after treatments were applied. The number of worms on 20 marked plants per plot was recorded as indicated. The damage estimate, made 2 weeks after treatment, is reported as per cent increase over the least damage treatment, which was Sevin. In 1958, six spray formulations were applied with high clearance equipment to four-row plots, 65 feet long. Fifteen undamaged plants in the center rows of each plot were marked and uniformity of infestation insured before treatment. In addition to the data on larval reduction, damage estimates were made 9 and 22 days after treatment, the second estimate being primarily an evaluation of the residuality of the treatments under the stress of continuous oviposition by a heavy wave of adult moths.

Residue Analysis.—The tobacco from the 1958 spray treatments mentioned in the paragraph above was sampled at various intervals for residue analysis. Ten random bottom leaves from the middle 2 rows of each plot were composited for each treatment at the intervals indicated in table 5. In the residue dissipation study the analyses were made on green tobacco. In these cases the leaves were primed, extracted, and the extracts stored

at −15° C. to await analysis. In addition to the studies made on the green tobacco, bottom-leaf samples from each treatment were primed 15 hours after treatment and cured separately in small compartment barns. After curing, 2-pound samples of the cured tobacco were made available for analysis.

The analytical method used for the determination of Guthion and oxy-Guthion was based on the procedure set forth by MacDougall (1958). Modifications in chromatographic separations permitted the determination of the total Guthion:oxy-Guthion complex and of Guthion alone. The determination of Thiodan residues was based on the procedure of Cassil (1958) as modified by Graham (1959). Residues of Sevin were determined using a modification of the procedure of Miskus (1958) and Whitehurst (1958). Determination of Dipterex residue toxins in aqueous extracts of green and cured tobacco followed the procedure of MacDougall (1956), which is a modification of the delta pH-cholinesterase method of Giang & Hall (1951). Apparent inhibition effects of the check tobacco extracts made it necessary to run a standard curve for each concentration of extract used. One sample from the SD 4402 15-hour treatment was flue cured and bioassayed[3] in accordance with the procedure of Sun & Sun (1952), using *Drosophila* as the test insect. Details on the analytical procedures used in the determination of Guthion:oxy-Guthion, Sevin, Thiodan, and Dipterex on green and flue-cured tobacco will be set forth in a future paper.

Flavor Tests.—The bulk of the tobacco primed and cured at the 15-hour interval from the 1958 insecticide plots was made into cigarettes and submitted to a cooperating manufacturing company's taste panel for flavor evaluation. The taste tests were similar to those described previously (Entomology Faculty 1958).

Insect Pathogens.—In 1957 *Bacillus thuringiensis* spore material was obtained from Dr. E. A. Steinhaus of the University of California. One lot of spores prepared in June 1956 and another prepared in March 1953 were used. Spore material of *B. sotto* Ishiwata was produced at North Carolina State College by Dr. A. E. Borg from a culture provided by Dr. Steinhaus. Triton B-1956 was used as a wetting-spreading agent at 1–2,000.

Three tests were made utilizing this material. In the first test, nine treatments were applied to single row plots, 45 feet long. Five uninjured plants in each plot of treatments 1, 2, 4, and 9 were staked and infested with four to five hornworms immediately before applications were made. In addition, one 4th or 5th instar larva was placed on each of the first 15 unstaked plants in all plots before treatments were applied. Pretreatment counts were made on staked but not on unstaked plants. Treatments were applied with a hand sprayer or a rotary hand duster. A damage evaluation of the staked plants was made 10 days after treatment.

In the second test in 1957, 10 uninjured and succulent plants were staked several hours in advance of treatment and infested with two 5th instar and one 4th instar tobacco hornworms. At the time of treatment the amount of leaf damage on each staked plant was estimated so that this damage could be subtracted from subsequent damage evaluations. The single-row, 65-feet-long plots were treated with a high-clearance sprayer. A damage estimate was made 1 week after treatment.

In the third test, 10 vigorous colonies of *Polistes exclamans* Vier. (one of the important predators of hornworms) established in wooden shelters were utilized. These were divided into two groups in such a manner as to obtain approximately equal numbers of the immature and adult stages in both groups. A pretreatment count of eggs, larvae, pupae, and adults was made in all colonies. One group of colonies was sprayed with a spore suspension of *B. thuringiensis* and the other group with water. A hand sprayer applied the spray solutions to the point of run-off.

In 1958, commercial preparations of *Bacillus* from two sources were tested. Bioferm spore material was designated as No. 57-18 Microbial Insecticide and had 16.6 billion viable spores per gram, *B. thuringiensis* PYP No. 504. Two sample lots, designated as S7-138 and S8-182, respectively, were used. Merck spore material was designated as MK-134 Microbial Insecticide, and the 1-pound lot (TN 47-109) received for testing contained 100 billion spores per gram. Treatments were replicated three times in Test 1 and four times in Test 2. Each plot consisted of two rows of tobacco 40 to 80 feet long, which were treated with a high-clearance sprayer. Counts of hornworms were made on 5 or 10 marked plants before, and at 2- and 3-day intervals after treatments were applied. Estimates of hornworm damage were made on each marked plant before and about 1 week after treatments were applied.

RESULTS.—*Laboratory Tests.*—In 1956, as shown in table 1, Guthion and DDVP were found to be appreciably more toxic, Thiodan less toxic, and Dipterex about equal to endrin in topical toxicity. In 1958, Shell SD 4402 was found to be nearly as toxic, and Sevin was considerably less toxic than endrin. Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate) Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), ethion, and Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate) lacked appreciable contact toxicity to tobacco hornworms.

FIELD TESTS.—*Insecticides.*—The tests in 1956 (table 2) were difficult to evaluate owing to variations in the amount of dust delivered. The 1:10 dust mixture (1%

[3] Bioassay analysis courtesy of J. E. Pankaskie, Shell Development Company, Modesto, California.

Table 1.—LD-50 values, micrograms/larva, of insecticides applied topically to 5th instar tobacco hornworms weighing 4 to 6 grams.

MATERIAL	PURITY	LD-50
1956		
Guthion	94%	61
DDVP	100%	77
Endrin	93%	131
Dipterex	99%	140
Thiodan	Tech.	650
Chlorthion	Tech.	>1,000
Delnav	Tech.	>1,000
1958		
Endrin	95%	95
Shell SD 4402	Tech.	140
Sevin	Tech.	800
Ethion	95%	>1,000
Trithion	95%	>1,000

Table 2.—Effectiveness of dust formulations in controlling tobacco hornworms. Clayton, N. C. 1956.

Treatment	Active Ingredient per Acre (Lb.)	Number of Hornworms[a] per 20 Plants				
		12 Hours After Treatment			44 Hours After Treatment	
		Dead	Living	Reduction Over Check (%)	Living	Reduction Over Check (%)
1% parathion+10% TDE	0.13	53	4	91	1	97
10% TDE	2.0	5	18	57	7	85
1½% endrin	0.2	38	12	70	6	87
5% Dipterex	0.55	62	6	86	4	91
3% Thiodan	0.63	28	10	76	6	87
Check	—	0	41	0	47	0
L.S.D.[b] at 5% level			6		5	
at 1% level			8		7	

[a] 3rd, 4th and 5th instar.
[b] Analysis did not include check data.

parathion+10% TDE) was faster after 12 hours than 10% TDE, 1½% endrin, or 3% Thiodan. Five per cent Dipterex was faster than TDE or endrin but was not significantly faster than Thiodan. Forty-four hours after treatment the 1:10 dust mixture was more effective than all treatments except Dipterex.

The results with the spray and dust formulations shown in table 3 indicate that endrin, Sevin, and Dipterex sprays are about equal in speed of action and in reducing hornworm damage. Both spray and dust formulations of Guthion were considerably less effective than the other treatments in controlling hornworms at the concentrations used. The amount of damage permitted by the treatments was in agreement with the control data.

Table 4 shows that all the materials were about equal in speed of action even though Thiodan and Shell SD 4402 appeared to be slightly inferior (but not at the 5% level) to the other materials. After 40 hours all the materials gave good to excellent results although Thiodan gave significantly poorer results than the other materials. Nine days after treatment there was no difference between treated plots in damage. At the 20-day damage evaluation, however, the Dipterex treatment had obviously dissipated, and the Guthion treatment, although permitting less damage than the check and Dipterex treatments, was also inferior to the other treatments.

Residues Analysis.—The residues found on the treatments discussed in the preceding paragraph are reported in table 5. As would be true of any crop with a large surface area and small volume the residues were initially quite high. The dissipation curves on green tobacco showed that Dipterex was the least persistent material. Sevin, Thiodan, and Guthion gave roughly the same results, being dissipated appreciably but having 10 to 16 p.p.m. 15 days after treatment. It should be emphasized that dissipation of the insecticides was due primarily to weathering. Growth dilution was not a factor since all leaves were taken from the lower part of the plant and were full-grown at the initiation of sampling.

When the treated tobacco was primed (15 hours after treatment) and flue cured (temperatures rose slowly over a period of several days to 160° to 170° F.), the residues from the Dipterex treatment had lost 99%, Sevin 89%, Guthion 91%, and Thiodan 86% of the amount found on green tobacco 1 day after treatment. As values for the green tobacco samples of SD 4402 are not available at this time, the per cent loss during flue curing cannot be stated. However, as only 1 p.p.m. was found on the cured leaf it is obvious that considerable dissipation had occurred.

Flavor Tests.—A report from a major tobacco company showed that cigarettes made from tobacco primed 15

Table 3.—Effect of insecticidal sprays and dusts on control of naturally heavy tobacco hornworm infestations and their damage. Greenville, N.C. 1957.

Treatment	Formulation[a]	Active Ingredient per Acre (Lb.)	Number Living Larvae on 20 Plants[b] after Hours Shown							Increase in Tobacco Foliage Consumed (%)
			Pre-treatment Instar			16	24	45	72	
			3	4	5					
Endrin	18½% E.C.	0.20	9	16	28	7	5	1	—	13
Guthion	2½% dust	0.88	6	11	29	36	31	19	12	65
Guthion	15% E.C.	0.20	12	18	27	24	19	13	7	68
Sevin	24% E.C.	1.00	6	10	30	6	4	2	—	0
Dipterex	50% S.P.	0.50	7	13	28	5	5	2	—	15
Check	—	—	7	10	30	42	36	24	18	156
L.S.D. at 5% level						9		5		
at 1% level						13		7		

[a] E.C., emulsion concentrate; S.P., soluble powder.
[b] Average of four replications.

Table 4.—Effect of insecticidal sprays in controlling tobacco hornworms and their damage. Clayton, N.C. 1958.

Treatment	Lb./Gal.	Active Ingredient per Acre (Lb.)	Hornworms (by Instar) on 15 Marked Plants[a] Before Treatment			Per Cent Reduction of 3rd, 4th, and 5th, Instars After		No. Leaves Lost/ 15 Plants on Indicated Days After Treatment[a]	
			3rd	4th	5th	16 hrs.	40 hrs.	9	22
Endrin	1.6	0.4	25	28	6	89	99	4	1
Thiodan	2	0.8	26	25	7	74	87	7	3
Guthion	1.5	1.0	23	24	8	83	96	5	16
Sevin	2.5	0.8	29	23	9	91	98	4	7
SD 4402	1.25	0.2	28	22	9	74	98	5	7
Dipterex	50%[b]	1.0	28	22	9	87	98	3	45
Check	—	—	32	25	6	0	+1	88	117
L.S.D. at 5% level						ns	6.0	ns	13
at 1% level						ns	8.0	ns	18

[a] Average of four replicates.
[b] Soluble powder.

Table 5.—Residues of insecticides found on green and flue-cured tobacco. Clayton, N.C. 1958.

Material	Dosage (Lb./A.)	P.p.m.[a] at Indicated Days Following Treatment						
		Green Tobacco[b]					Cured Tobacco	Loss During Curing (%)
		1	4	8	11	15	1	
Thiodan	0.8	96	34.2	20.3	21.3	16.6	12.7	86
Guthion	1.0	356	34.0	24.8	20.2	13.8	31.1[c]	91
Sevin	0.8	132	29.4	12.8	10.1	10.1	13.7	89
Dipterex	1.0	213	9.4	2.5	1.1	1.3	0.1	99
SD 4402	0.2	—	—	—	—	—	1.0	—

[a] Dry, stemless basis.
[b] Rainfall at indicated days after treatment: 1.73 in. 5 days, 0.58 in. 13 days, 0.36 in. 14 days.
[c] Guthion-Oxy-Guthion; green tobacco, Guthion only.

Table 7.—Effect of endrin and *B. thuringiensis* on tobacco hornworms and their feeding. Clayton, N.C. 1957.

Material	Rate/ Acre	Living Hornworms[a] After Hours Shown			Leaves Lost[a]
		24	48	72	
Endrin (19½% E.C.)	0.3 lb.	0.2	0.0	0.0	3.6
B. thuringiensis (old)	68 gm.	19.2	5.6	1.8	5.8
B. thuringiensis (old)	204 gm.	14.6	1.6	0.6	3.2
Check (water)	40 gal.	29.8	22.6	15.0	74.0
L.S.D. at 5% level		5.2	2.0	2.5	5.6

[a] Average per 10 plants.

hours after treatment showed no effect on taste or flavor except where Sevin was used. In this case a slight off-flavor was reported.

Insect Pathogens.—In the test reported in table 6, the insecticides were much quicker in action than the pathogens and gave a greater reduction in living larvae within 72 hours after treatment. Leaf loss to hornworms, however, was about the same in plots receiving 96 grams per acre of *B. thuringiensis* (treatment 4) as in those treated with endrin and TDE (treatments 1 and 2). A difference in effectiveness between the 1953 and 1956 lots of spores from California was not demonstrated. Since the 64 gram-rate of *B. sotto* performed as well as the highest (163 grams) and most effective rate of *B. thuringiensis*, it seems probable that the former is more toxic to hornworms.

In another test (table 7) endrin again proved more rapid in action than *B. thuringiensis* but failed to give a greater reduction in damage. Also, no significant difference occurred in the performance of the 68- and 204-gram rates of *B. thuringinesis*.

Counts of each stage of *Polistes exclamans* before and after treatment with *B. thuringiensis* and water are summarized in table 8. There was no indication that the

Table 6.—Effectiveness of *Bacillus thuringiensis*, *B. sotto*, and several insecticides in controlling tobacco hornworms. Clayton, N.C. 1957.

Treatment	Formulation	Rate/Acre[a]	Average No. of Living Larvae per Plot After Hours Shown			X+0.5 72 Hrs.	Leaves Lost per 5 Plants
			24	48	72		
1. Endrin	19½% E.C.	0.45 lb.	0.3	0.0	0.0	0.71	3.0
2. TDE	25% E.C.	1.10 lb.	4.3	0.3	0.0	0.71	4.0
3. *thuringiensis*	Calif., 1956	42 gm.	14.8	12.5	7.3	2.56	—
4. *thuringiensis*	Calif., 1956	96 gm.	14.5	12.0	5.3	2.37	2.5
5. *thuringiensis*	Calif., 1953	87 gm.	17.0	9.0	3.3	1.90	—
6. *thuringiensis*	Calif., 1953	163 gm.	10.3	7.0	2.3	1.60	—
7. *sotto*	N.C., 1957	64 gm.	12.0	8.5	2.3	1.60	—
8. Guthion	2½% dust	1.30 lb.	3.8	2.3	1.0	1.19	—
9. Check	—	—	20.0	15.0	12.8	3.61	42.2
L.S.D. at 5% level						0.80	
at 1% level						1.09	

[a] Active ingredient for insecticides and weight of dry spore powder for pathogens.

Table 8.—Effect of *B. thuringiensis* sprayed directly on colonies of *Polistes exclamans*. Clayton, N.C. 1957.

Treatment	Number[a] of Each Stage of *Polistes* Before and on Days After Treatment Shown															
	Eggs				Larvae				Pupae				Adults			
	Pre-Treat	3	7	14	Pre-Treat	3	7	14	Pre-Treat	3	7	14	Pre-Treat	3	7	14
B. thuringiensis 2 gm./gal.	62	81	71	67	177	158	158	98	177	159	219	291	66	52	73	34
Check (water)	73	61	73	54	162	133	143	151	134	185	245	287	54	53	77	35

[a] Total number of each stage in five colonies of wasps.

Bacillus was harmful to this important hornworm predator.

Two tests of commercial preparations of *B. thuringiensis* yielded the data presented in table 9. In test 1, the 282 grams of Bioferm and 188 grams of Merck spores brought about a significantly higher reduction in larvae than the two lower rates of Bioferm spores. On the basis of damage, no treatment was significantly better than the others, though all were better than the check. In test 2, the 47-gram dosage of Merck spores was significantly poorer than the other treatments in reducing larval populations and damage. The 188 grams of Merck and Bioferm spores produced comparable results. The 752 grams of Merck material gave the highest reduction in hornworms and resulted in the least damage but was not significantly (5% level) better than the 188 grams.

CONCLUSIONS.—Certain insecticides tested showed considerable promise for hornworm control. In addition, they had a distinct advantage for future planning of tobacco insecticide-control programs in that the insecticide residues were drastically reduced or eliminated during curing. Further work needs to be done to determine the fate of these insecticides during smoking (heat degradation at 800° C.) and to determine their effectiveness in controlling the other major insect pests of tobacco in advanced testing programs.

Bacillus thuringiensis spores were found to be as effective as recommended insecticides in preventing damage by hornworms but were slower in their action. It would appear that spore-treated worms stop feeding soon after ingesting spores but remain in an apparently healthy condition for 2 or more days. *B. sotto* appears to be somewhat more effective than *B. thuringiensis* in reducing hornworm populations. Commercial preparations of *Bacillus* gave good control of hornworms. Results in other tests have indicated that the *Bacillus* treatment is not effective against the tobacco budworm, *Heliothis virescens* (F.), and this fact greatly reduces its potentiality for tobacco insect control.

Table 9.—Effectiveness of commercial preparations of *Bacillus thuringiensis* spores against tobacco hornworms. Clayton, N.C. 1958.

Test No.[a]	Source of Spores	Rate per Acre (Grams)	Total No. of Larvae Before Treatment	Mean Reduction Larvae After 72 Hours		Mean Number of Leaves Lost per 10 Plants
				(%)	Angles[b]	
1	Bioferm	47	167	79	58	32.5
	Bioferm	141	145	82	61	12.2
	Bioferm	282	170	97	79	1.3
	Merck	188	171	94	73	10.5
	Check	—	194	23	—	99.8
L.S.D. at 5% level					9	32.1
2	Merck	47	233	69	52	35.3
	Merck	188	212	89	73	13.3
	Merck	752	217	94	74	3.8
	Bioferm	188	203	84	63	13.2
	Check	—	227	18	—	153.7
L.S.D. at 5% level					15	17.8

[a] Tests 1 and 2 were each replicated three times. Counts were made on 10 plants per plot.

[b] Reduction percentages corrected by Abbott's formula and transformed to angles.

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Control of *Sanninoidea exitiosa graefi* (Hy. Edw.) on Apricots

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ABSTRACT

Studies were conducted on the control of the western peach tree borer, *Sanninoidea exitiosa graefi* (Hy. Edw.), in apricots with both soil fumigants and trunk sprays from 1955 through 1958. Results were first evaluated by caging the trees to trap emerging adults, later by counting empty cocoons or pupal cases, and finally by strike counts in the early fall. The last-mentioned method was found to be the most practical.

In the soil fumigation trials paradichlorobenzene, propylene dichloride, and tetrachloroethane gave good results. Ethylene dichloride was the least effective in these trials. Tetrachloroethane, although it killed borers, was extremely phytotoxic.

Trunk treatments were made during the 1957 and 1958 seasons. In 1957 Thiodan® (6,7,8,9,10-hexachloro-1,5,5*a*,6,9,9*a*,hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and endrin applied three times at monthly intervals gave good control. DDT was also effective, and there was little difference between treatments at monthly or bimonthly intervals. The addition of a sticker to DDT did not improve its performance. Whitewash, applied to the trees in late May, did not prevent borer attack. In 1958, Thiodan and endrin gave excellent control whether treated four times at monthly intervals or twice at bimonthly intervals. Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) was effective at monthly intervals but less so at bimonthly intervals. DDT was not so good as the above compounds, but gave commercial control when applied four times at monthly intervals. A single treatment of dieldrin in June did not provide satisfactory control.

The western peach tree borer, *Sanninoidea exitiosa graefi* (Hy. Edw.), is an important pest of stone fruits in California. Although Essig (1958) lists peach as the perferred host, the insect is most commonly found on apricots in northern California. Plums, prunes, cherries, almonds, and peaches are all attacked, but the most serious damage is to apricots.

Its importance on apricots is probably due to the geographical location of the stone fruits in California. Most of the peaches are grown in the interior valleys of California, where the western peach tree borer is not an important pest. It is most serious in the coastal districts where the majority of the apricots and but few peaches are grown. It is most important in the counties located near the San Francisco bay area, which contains over half of the State acreage of apricots. It is in this area that western peach tree borer is most serious.

One of the problems associated with the control of this insect is the long emergence period of the adults. This has been pointed out in studies on the peach tree borer in the Southern States, where Snapp & Thomson (1943) found that the moths emerge from May to November with a peak in August and September. King & Morris (1956) reported that in Texas the moths emerge from late May to October, with a decline in August followed by a peak in September.

During the course of investigations in California it was determined that the moths of the western peach tree borer first appear in late April or early May, and continue through September. The peak emergence according to data from several seasons is in July or early August. The periods of lowest adult emergence are usually in May or September, but on occasion a period of hot weather in May will bring about a moderately heavy emergence.

Control studies on this pest were begun in 1955 and continued through the 1958 season.

MATERIALS AND METHODS.—The first control experiments during the 1955 and 1956 seasons were conducted with soil fumigants. They were applied to the trees by various methods, depending upon the nature of the compounds. Tests during 1957 and 1958 were with trunk sprays, and the materials were applied with a conventional high-pressure rig and orchard spray guns. The pressure was reduced to 200 p.s.i. in order to avoid excessive splashing of the materials.

One of the major problems associated with experimental work on the western peach tree borer is the evaluation results. In the early experiments treatments were made in one season and evaluated the following year by emergence records. At first cages were constructed around the base of each tree to trap the emerging moths. This practice did not prove feasible, as the moths were difficult to see, weed growth within the cage area was hard to remove, and many cages were damaged by cultivation and irrigation practices.

It was subsequently ascertained that a record of emergence could be made by counting the cocoons and pupal cases around the trees at intervals throughout the season. It was necessary to remove weeds and smooth the ground around the trees to make this method feasible. Since the

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cocoons and pupal cases remain projecting from the tree trunk or from cracks in the soil, they are relatively easy to count. Although this method was satisfactory it was still necessary to evaluate results the year after treatment.

During the 1957 season it was noted that the attack of the borers in late summer was characterized by the presence of a small area of frass on the tree trunk. Frass areas were usually scattered over the trunk above the soil line, with each area indicating the work of a single borer. These spots, which will be referred to as "strikes," were counted on the test trees during the fall of 1957, and the same trees were checked for adult emergence the next year. The two methods of evaluation correlated very well and the strike count was adopted for the 1958 trunk trials. It was necessary to make these counts in early fall, as the frass areas tended to gum heavily and coalesce into large areas by late October and November. It was then impossible to determine how many borers were involved. In the spring masses of jellylike gum were present on the injured areas, so that it was still impossible to determine from an external examination how many borers were present.

SOIL FUMIGANT TRIALS—1955–1956.—The standard treatment in California for western peach tree borer control is the use of paradichlorobenzene (PDB) as a fumigant in the fall. In order to test this standard treatment in comparison with other fumigants, a test plot was established in a young Blenheim apricot orchard which showed evidence of heavy borer attack. Ethylene and propylene dichloride were included as Snapp (1950) and Anthon (1955) have reported that these compounds gave good control. Tetrachloroethane was also used since the compound was being used for borer control.

The most heavily infested trees in the orchard were selected for the trials, and each treatment was applied to six trees in an unrestricted randomized plot design. Materials were applied on November 2. PDB was used at the rate of 1 ounce per tree and was applied as a band 2 inches from the trunk. The crystals were covered with earth and packed down with a shovel. Ethylene and propylene dichloride were diluted using an emulsion of 6 parts of water to 4 parts of 50% emulsion. They were poured around the trees at the rate of one-half pint per tree. The treated area was then covered with dirt and packed down. Tetrachloroethane was injected with a weed gun set to deliver one-half ounce of undiluted material per injection. Eight injections were made around each tree to give a 4-ounce-per-tree dosage.

In addition to the above compounds, exploratory trials with two trees in each instance were made with Vapam® (sodium methyldithio-carbamate), Nemagon (1,2,Dibromo-3-chloropropane), Stauffer 339, parathion granules, and lindane drench. The plots were limited since nothing was known concerning the phytotoxic properties of these compounds.

The plots were evaluated the following season by counting cocoons and pupal cases. The data are summarized in table 1. PDB, propylene dichloride, and tetrachloroethane gave satisfactory results as compared with the check plots. Ethylene dichloride was not so effective as the other materials.

No counts were made on the exploratory compounds since so few trees were involved in the trials. Observations showed, however, that borers were present in the treated trees, and none of the compounds indicated any outstanding degree of control.

Table 1.—1955 soil treatments for control of the western peach tree borer.

MATERIAL[a]	DOSAGE PER TREE	TOTAL MOTH EMERGENCE	AVERAGE EMERGENCE PER TREE
Paradichlorobenzine	1 oz. of crystals	8	1.3
Propylene dichloride	½ pint of a 6-4 dilution	7	1.1
Ethylene dichloride	½ pint of a 6-4 dilution	24	4.0
Tetrachloroethane	4 oz. in eight ½ oz. injections	4	0.8
Check	No treatment	44	8.8

[a] Application made on November 2.

It was noted that the trees treated with tetrachloroethane showed adverse effects, but since they had been so badly damaged by borers it was not possible to ascertain the true effect of the chemical. To further check this point, tetrachloroethane, along with ethylene and propylene dichloride, were applied in the spring of 1956 to a healthy young almond orchard on apricot root stock. Five trees were used per treatment, and the compounds were applied in the same manner as previously described. The trees were examined throughout the season, and by summer three of the five trees treated with tetrachloroethane were dead. The other two showed marginal leaf burn, poor growth, and shriveled nuts. The trees did not show any sign of injury from ethylene and propylene dichloride. The adverse effects of tetrachloroethane were noted on grower-applied plots in addition to these trials; therefore it was concluded that this compound is too phytotoxic for use on fruit trees.

TRUNK TREATMENTS 1957–1958.—DDT has been used as a trunk spray by growers in California for western peach tree borer with varying results. The long emergence period of the adult makes it necessary to make several applications during the season. The number of treatments needed seems to vary, according to the reports of other workers. Martin (1953) reported the three applications of DDT, lindane, or dieldrin gave satisfactory results in Texas. McCreary (1954) in Delaware states that five applications of DDT, BHC, dieldrin, parathion, and EPN gave control, but three applications with a sticker were slightly less effective. Snapp (1958) reported that trunk sprays of DDT, BHC, and parathion were effective against light infestations, but were not dependable against a heavy infestation.

Since the results with DDT in California were so variable a plot was established in the 1957 season on a mature Blenheim apricot orchard which was heavily infested with western peach tree borer. DDT was used at monthly and bimonthly intervals, with and without a sticker. Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2-4-3-benzodioxathiepin-3-oxide) and endrin were also used at monthly intervals. Whitewash had been reported by growers to reduce borer attack, and was included in the trials. Each material was applied to four trees with three replications in a randomized block de-

Table 2.—1957 trunk sprays for control of the western peach tree borer.

Materials	Dosage per 100 Gallons	Total "Strikes" per 12 Trees (Sept. 1957)	Total Adult Emergence 1958
Check	no spray	44	35
DDT	8 lb. WP 50%[a]	10	7
DDT	8 lb. WP 50%[b] plus 1 qt. National sticker	12	8
DDT	8 lb. WP 50%[b]	7	5
Endrin	2 qt. EC 1.6[ac]	0	0
Thiodan	8 lb. WP 25%[a]	0	0
Whitewash	—[d]	—	14
Check	no spray	32	28

[a] Applied May 29, June 28, August 2.
[b] Applied May 29, August 2.
[c] Formulation contained 1.6 lb. endrin per gallon.
[d] Applied May 29.

Table 3.—1958 trunk sprays for control of the western peach tree borer.

Materials	Dosage per 100 Gallons[a]	Average "Strikes" per 12 Trees	Average No. "Strikes" per Tree
Thiodan	1 gal. EC, 2.0[b]	0	0.0
Thiodan	1 gal. EC, 2.0[c]	1	0.1
Check	—	79	6.5
Endrin	2 qt. EC, 1.6[b]	0	0.0
Endrin	2 qt. EC, 1.6[c]	0	0.0
Check	—	82	6.8
Guthion	8 lb. WP 25%[b]	1	0.1
Guthion	8 lb. WP 26%[c]	12	1.0
DDT	8 lb. WP 50%[b]	14	1.1
Dieldrin	1 gal. EC, 1.5[d]	25	2.0

[a] Emulsion concentrates contained pounds of active ingredient per gallon as follows: Thiodan, 2; endrin, 1.6; dieldrin, 1.5.
[b] Applied May 15, June 18, July 25, Aug. 18.
[c] Applied May 15, July 25.
[d] Applied June 18.

sign, using the spray equipment previously described. Before application the weeds were removed from around each tree. The sprays were applied to the trunk, from the main limbs to the soil line, and an average of 1½ gallons was applied per tree. The first treatments were made in May, and subsequent sprays were at monthly or bimonthly intervals. The plots were evaluated in the fall of 1957 by making counts of the "strikes" on each tree. To check this method of evaluation, the pupal cases and cocoons were examined the following season. The data are summarized in table 2.

In all cases the emergence counts correlated with the strike counts, but the total emergence was always less than had been recorded in the fall. This can be explained by natural mortality and by the probability that some cocoons and pupal cases are lost during orchard disking and irrigation. The 1957 data showed that Thiodan and endrin were very promising for borer control. There was little difference between the DDT plots, and all gave what could be considered commercial control. Whitewash was not an effective means of preventing borer attack.

In the 1958 season trunk spray trials were continued to further evaluate Thiodan and endrin. Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) was also included as other phosphates have been reported to give control of peach tree borer. Smith (1952) has stated that parathion and EPN give good control, apparently because of ovicidal action. A treatment of a single heavy dosage spray of dieldrin was used as Wylie (1956) reported that a single application of dieldrin in June resulted in seasonal control.

During the 1957 season it had been observed that trees that showed evidence of a prior heavy borer attack were more susceptible to reinfestation than trees with only light attack. Therefore, the trees used in the 1958 trials were selected on the basis of having a minimum of four areas of gum and frass per tree. Twelve trees that met the above qualifications were selected and marked from single tree rows for each treatment. Check rows with similarly selected trees were left between the treated rows. Each material was used at monthly and bimonthly intervals with the exception of DDT, which was used at monthly intervals only, and the single application of dieldrin. The first sprays were applied on May 15, as a period of warm weather in late April was followed by an adult emergence in early May. The plots were evaluated in early September by making strike counts on the treated and untreated trees. The 1958 data are summarized in table 3.

Thiodan and endrin gave excellent control at both monthly and bimonthly intervals. Guthion was effective when used at monthly intervals, but was less effective at bimonthly intervals. DDT, although not so good as the above compounds, gave what could be considered commercial control. The single treatment of dieldrin, although reducing the number of bores as compared with the check, was not satisfactory. The performance of Thiodan and endrin indicates that it may be possible to control western peach tree borer with but two applications per season.

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Response of Cotton and Cotton Pests to Thimet Seed-Treatment[1]

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Abstract

Coker 100W cottonseed treated with Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) was evaluated in replicated field tests at four widely separated locations in North Carolina during 1955–56. Thimet exhibited pronounced effects both upon the cotton plant and pests which attack cotton. Thimet seed-treatments were particularly effective in controlling thrips, *Frankliniella fusca* (Hinds), *F. exigua* Hood, *F. tritici* (Fitch), *Thrips tabaci* Lind., and the cotton aphid, *Aphis gossypii* Glov., until the time of squaring. This material afforded seasonal protection from spider mites, *Tetranychus cinnabarinus* (Bois.) and *T. telarius* (L.). Thimet was partially effective in reducing boll weevil, *Anthonomus grandis* Boh., infestations throughout the season, but the use of this material favored the development of bollworm, *Heliothis zea* (Boddie), and tobacco budworm, *H. virescens* (F.), infestations. Delayed fruiting of cotton appeared to be the most detrimental characteristic associated with the use of Thimet. However, certain adverse effects upon the germination, emergence, and vegetative growth of cotton were also noted.

The systemic insecticidal efficiency of Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) as a seed-treatment for cotton was first reported by U. S. Department of Agriculture workers at College Station, Texas, in 1954 (Anonymous 1954). The results of these studies were published by Ivy *et al.* (1957). In laboratory tests these workers found that 4 pounds of Thimet per 100 pounds of cotton seed produced no plant injury. At this dosage Thimet effectively controlled boll weevils, cotton aphids, spider mites, and thrips for 7 to 9 weeks but failed to control bollworms. Thus, Thimet was shown to be particularly promising for control of four of the five major pests of cotton in North Carolina. In addition to the simplicity of application the use of an effective systemic insecticide for early-season insect control could conceivably reduce the required number of mid- and late-season conventional insecticide applications.

Under North Carolina conditions the ecological and cultural factors affecting cotton favor a relatively short growing season. During most seasons cotton is planted early throughout the State within a 4-week period. The major portion of the crop is produced during the first 5 weeks of fruiting. Therefore, it is most profitable to begin insect control early for maximum early yields.

Investigations conducted in other states on the use of Thimet seed-treatment for cotton have been reported by Parencia *et al.* (1957a, 1957b), Reynolds *et al.* (1957), Hopkins *et al.* (1958), Hanna (1958), Dobson (1958), and Adkisson (1958). The results presented herein are in substantial agreement with those of the authors cited. However, the results of this investigation indicated a superior degree of insect control and more adverse effects on the cotton plant's capacity to produce an early crop of normal yield.

Methods and Materials.—Field-test data were obtained during 1955 and 1956 on the efficiency of Thimet seed-treatments for the control of cotton insects and the effect of the treatments on cotton plants. In all tests Thimet treatments were compared with toxaphene treatments and an untreated check.

Experiments 1 and 2, conducted in 1955 and 1956 respectively, were designed to determine the effect of the treatments on early-season cotton insects. Experiment 3 was designed to determine the seasonal effect of treatment on the boll weevil (*Anthonomus grandis* Boh.) and bollworm (*Heliothis zea* (Boddie)) in 1956. These field tests were located in areas representative of the wide range in environmental and cultural conditions existing in the State.

Experiment 1 was conducted at four locations. In Cleveland and Union Counties, located in the Southern Piedmont, the plots were on red sandy clay loam and brown sandy clay loam, respectively. In Scotland County, located in the Southern Coastal Plain, the tests were on brown sandy loam, and in Halifax County, in the Northern Coastal Plain, on brown fine sandy loam. Experiment 2 was conducted in Johnston County, in the Central Coastal Plain, on brown sandy loam, and in Union County. Experiment 3 was conducted in Cleveland and Scotland Counties. All fields received a complete fertilizer at the time of planting. In addition, fields in the Coastal Plain counties received a side-dressing of nitrogen at the time of thinning.

A randomized block design was used in each experiment. In experiment 1 a block of three randomized plots was located on each of three farms in each county. Each plot was approximately one-third acre. Experiments 2 and 3 consisted of four randomized plots within each of four adjacent blocks. Each plot was one-tenth acre in experiment 2, and one acre in experiment 3.

All test fields were planted between April 12 and May 12 at the rate of approximately 30 pounds of seed per acre. Planting was accomplished with either mule-drawn, single-row planters or with tractor-mounted, two-row planters. Some adjustment of the mule-drawn planters was necessary to increase the flow of seed when Thimet-treated seed was planted. In all tests Coker 100W variety was used except in two blocks in Scotland County, experiment 1, where one was planted with All-In-One variety and one with Moore's Special variety. In the 1955 tests the cotton seed of each cooperating grower was used. Most seed was reported to be either certified or registered, mechanically delinted, and treated with a fungicide. All 1956 tests were planted to the same lot of registered Coker

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100W seed which had been mechanically delinted and treated with a fungicide.

Thimet, formulated with activated carbon, was applied to the seed used in planting the Thimet-treated plots. In experiment 1 treatment was applied immediately before planting by tumbling the seed in a cardboard drum with Thimet at the rate of 4 pounds of 50% technical material per 100 pounds of seed. In experiments 2 and 3 seed was commercially-treated at the rates of 4 and 8 pounds of 44% Thimet per 100 pounds of seed.

Early-season treatments of toxaphene (*e.g.*, treatments started during the 2-leaf stage of cotton) were applied at weekly intervals to the toxaphene plots in experiments 1 and 2. Four applications of 10% toxaphene dust at 15 pounds per acre were applied with a rotary hand duster. All plots in experiment 1 and 2 received late-season applications of insecticides. In experiment 1 late-season insect control was performed by cooperating growers. In experiment 2 late-season insect control was obtained with applications of 3 to 4 pounds of toxaphene per acre at 5-day intervals until the plants in the Thimet-treated plots matured. In experiment 3 the toxaphene plots received the first application 1 week prior to squaring. Additional applications of 20% toxaphene dust at 20 pounds per acre were made at 5-day intervals until the plants matured. Other than the initial seed treatment the Thimet plots in experiment 3 did not receive further insecticidal treatment.

RESULTS AND DISCUSSION.—A reduction in plant emergence was apparent in some experiments during both years where Thimet seed-treatments were used (table 1). However, in no experiment were the reductions sufficiently large to affect the cotton stand following the thinning operation. Some of the differences in emergence can be attributed to erratic planting; however, records of planting rates and the trend in data indicate that other factors were involved. Adverse climatic and cultural conditions appeared to be more detrimental to the germination and emergence of Thimet-treated seed than of untreated seed. There was generally a short delay in the emergence and initial growth of seedlings from treated seeds. Speckling of the first leaves was also noticeable in most Thimet plots. Cotton plants in the Thimet-plots had luxuriant foliage in the late seedling stage, which diminished gradually as the season progressed. It did not appear that the luxuriant vegetative growth could be attributed solely to the control of insects.

Table 1.—Emergence of cotton plants in field tests for control of insects. North Carolina, 1955–56.

County	Number of Plants per 100 Linear Feet[a]			
	Check	Thimet, 0.5 Lb.	Thimet, 1.0 Lb.	L.S.D. 5%
	Experiment 1			
Cleveland	344	222	—	n.s.
Union	719	459	—	n.s.
Scotland	481	554	—	n.s.
Halifax	437	288	—	n.s.
	Experiment 2			
Union	225	230	238	n.s.
Johnston	660	471	421	n.s.
	Experiment 3			
Cleveland	445	463	417	19
Scotland	395	365	261	46

[a] Twenty-five consecutive feet on each of four adjacent rows sampled per replicate.

Table 2.—Thrips infestation in field Experiments 1 and 2. North Carolina, 1955–56.

Treatment[a] (Lb./A.)	Number of Thrips per Plant[b] On Weeks Following 2-Leaf Stage					Seasonal Average
	0	1	2	3	4	
	Experiment 1 Cleveland County					
Check	3.4	8.9	4.7	7.1		6.0
Toxaphene, 1.5	2.9	0.7	0.8	0.0		1.1
Thimet, 0.5	0.1	0.2	1.4	1.5		0.8
L.S.D. @ 5% level						4.6
	Union County					
Check	10.1	6.3	6.4	9.0		7.9
Toxaphene, 1.5	7.2	1.4	0.1	0.5		2.3
Thimet, 0.5	0.0	0.5	0.8	1.5		0.8
L.S.D. @ 5% level						2.6
	Scotland County					
Check	2.6	3.1	4.9	6.8		4.4
Toxaphene 1.5	2.6	1.1	1.5	0.8		1.5
Thimet, 0.5	0.1	0.1	0.1	1.1		0.4
L.S.D. @ 5% level						2.2
	Halifax County					
Check	2.1	2.8	1.9	2.1		2.2
Toxaphene, 1.5	2.2	1.2	0.0	0.0		0.8
Thimet, 0.5	0.0	0.0	0.0	0.0		0.0
L.S.D. @ 5% level						2.0
	Experiment 2 Union County					
Check	11.9	17.8	12.0	4.3	1.0	9.3
Toxaphene, 1.5	13.1	2.0	2.6	0.3	0.5	3.7
Thimet, 0.5	0.0	0.4	1.0	0.9	0.4	0.6
Thimet, 1.0	0.0	0.0	1.2	1.9	0.6	0.7
L.S.D. @ 5% level						3.2
	Johnston County					
Check	0.1	0.3	0.8	0.6	0.5	0.6
Toxaphene, 1.5	0.2	0.1	0.3	0.2	0.1	0.3
Thimet, 0.5	0.0	0.0	0.0	0.0	0.5	0.2
Thimet, 1.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D. @ 5% level						0.2

[a] Fifteen pounds per acre of 10% toxaphene dust at weekly intervals beginning at 2-leaf stage; pounds per acre of Thimet as seed treatments.

[b] Twenty plants sampled at random per replicate during the first 2 weeks, and 10 plants sampled during the remaining weeks.

The use of Thimet seed-treatment consistently delayed blooming of cotton during the first half of the normal fruiting period (figures 1 and 2). This resulted in delayed maturity of the crop (table 5). Thus, a large portion of the crop in the Thimet plots may be susceptible to high populations of late generations of the boll weevil and the late brood of bollworms. Furthermore, adverse climatic conditions are quite common during late-season in North Carolina, especially in the Coastal Plain counties.

Thimet seed-treatments effectively controlled thrips, *Frankliniella fusca* (Hinds), *F. exigua* Hood, *F. tritici* (Fitch), and *Thrips tabaci* Lind., on cotton throughout the period of thrips activity (table 2). This period extends from the emergence of seedlings through the eight-leaf stage. Thrips reproduction was not observed on cotton seedlings following the use of Thimet. Adult thrips found on plants in the Thimet-treated plots undoubtedly were transient forms. Plant height measurements, taken from the ground to the top of the terminal bud, were made at the eight-leaf stage (table 3). In most tests the untreated plants were shorter than plants receiving toxaphene or Thimet treatments. The greatest differences in height between treated and untreated plants were related to the higher thrips infestations.

Light infestations of the cotton aphid, *Aphis gossypii* Glov., and spider mites, *Tetranychus telarius* (L.) or *T.*

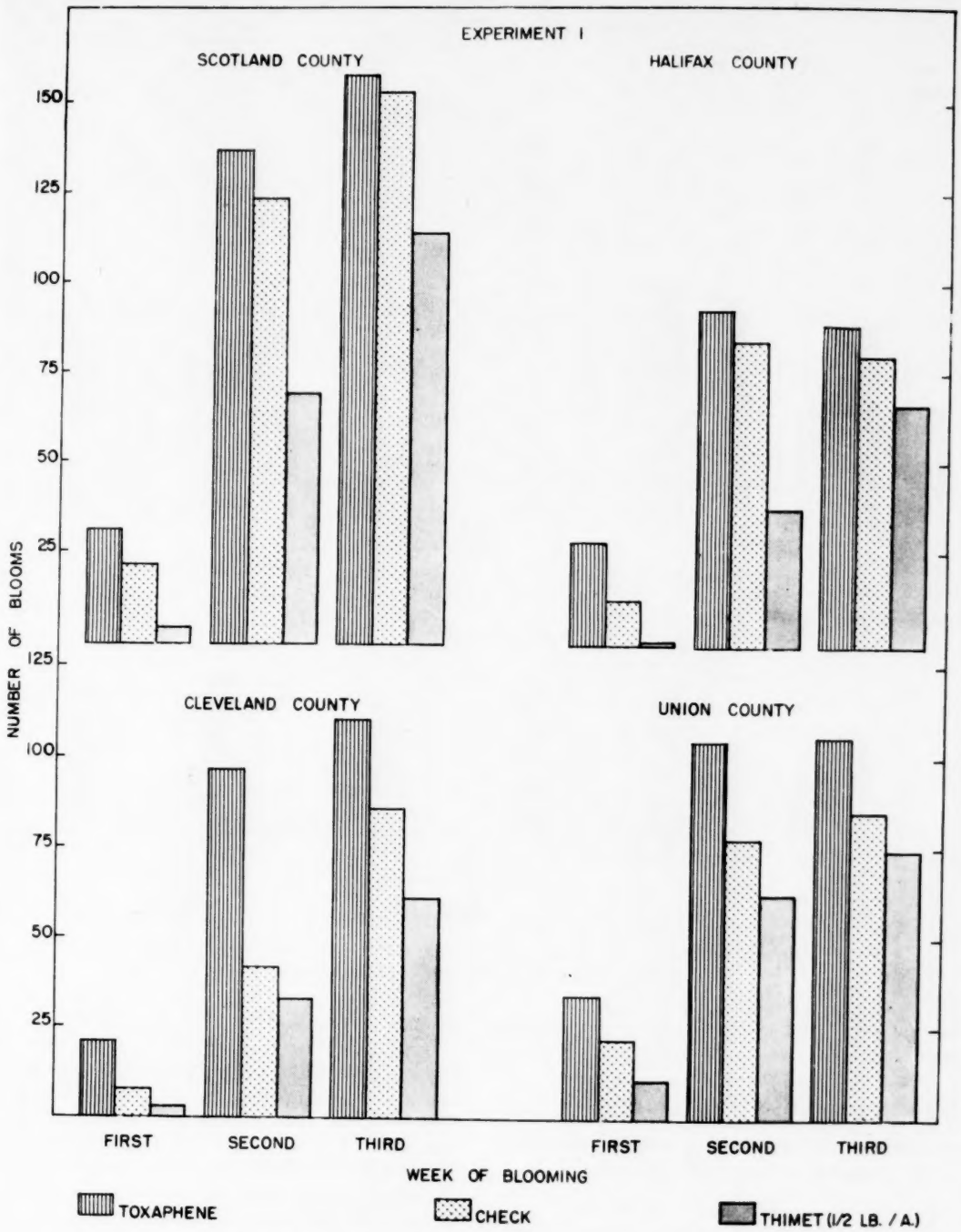

FIG. 1.—Average number of white blooms per 100 linear feet recorded on 1 day during the middle of each of the first 3 weeks of blooming.

cinnabarinus (Bois.), occurred early in the season in most tests. Thimet seed-treatments protected cotton plants from these infestations. In addition, Thimet afforded seasonal protection against heavy infestations of spider mites. In experiment 2 spider mites were active throughout the season at both locations. Demeton spray was used to control spider mites in the toxaphene and check plots in Union County whereas treatments were not applied for spider mite control in Johnston County. It was apparent that both levels of Thimet effectively controlled *T. cinnabarinus* in Johnston County and *T. telarius* in Union County throughout the season.

A reduction in boll weevil activity occurred throughout the season in plots treated with either ½ or 1 pound of Thimet (table 4). Under field conditions Parencia *et al.* (1957a) determined that Thimet was toxic to over-

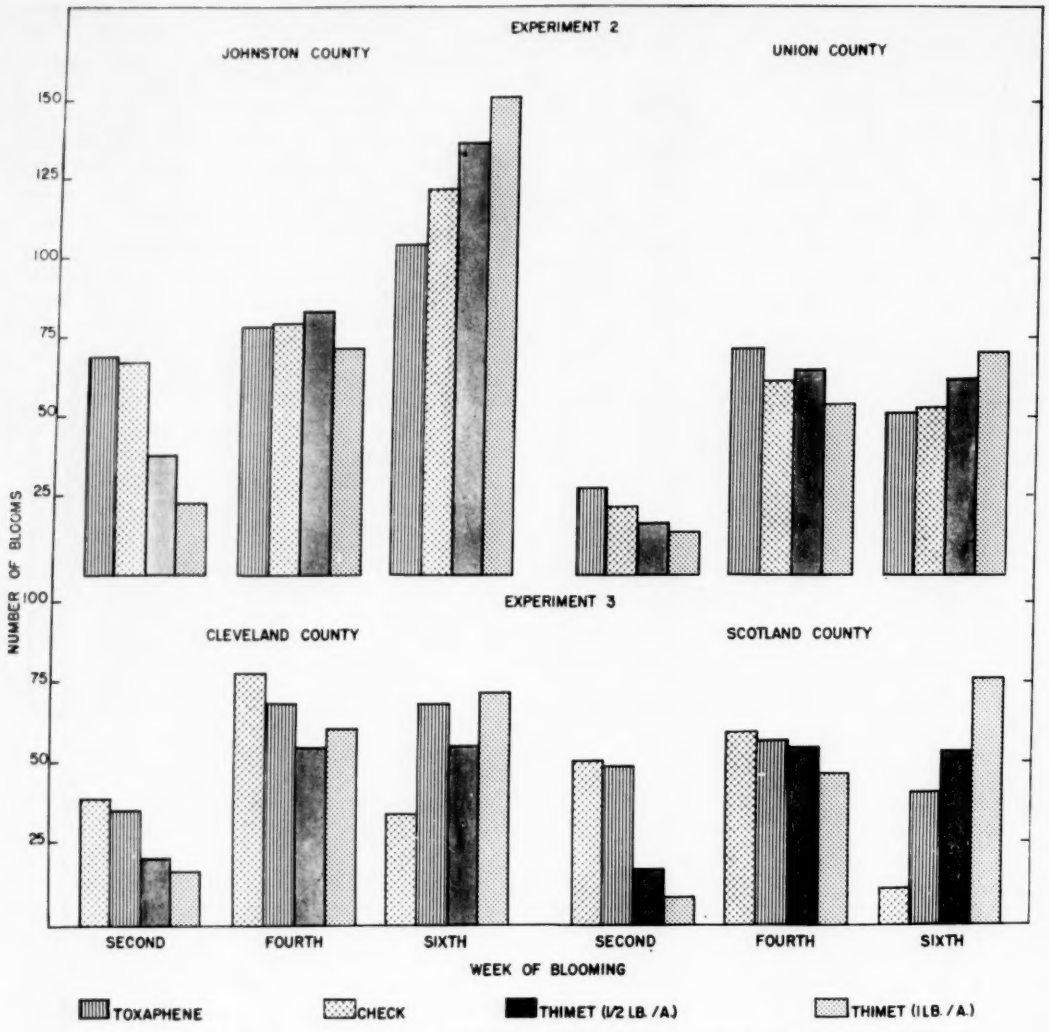

FIG. 2.—Average number of white blooms per 100 linear feet recorded on 1 day during the middle of the second, fourth, and sixth week of blooming.

wintered boll weevils for about 4 weeks following plant emergence. Ecological investigations in North Carolina have shown that throughout the season boll weevils tend to congregate on cotton with the greatest fruiting vigor. In the Thimet plots fruiting was retarded during the first half of the season and accelerated during the second half (figure 2). The trend in infestation data (table 4) suggests that both the insecticidal activity and different fruiting response due to Thimet should be considered in evaluating the effectiveness of this material for boll weevil control.

Table 3.—Height of cotton plants in field tests for thrips control. North Carolina, 1955–56.

County	Height of Plants in Inches at 8-Leaf Stage				
	Check	Toxaphene, 1.5 lbs.	Thimet, 0.5 lb.	Thimet, 1.0 lb.	L.S.D. 5%
		Experiment 1[a]			
Cleveland	8.7	11.7	10.5	—	n.s.
Union	6.9	9.0	9.3	—	1.2
Scotland	7.7	7.7	7.7	—	n.s.
Halifax	7.7	8.6	8.9	—	n.s.
		Experiment 2[b]			
Union	8.4	10.9	10.6	9.6	n.s.
Johnston	6.8	7.9	7.6	7.3	n.s.

[a] Five consecutive plants on each of four adjacent rows sampled per replicate.
[b] Twenty-five consecutive plants on each of four adjacent rows sampled per replicate.

Bollworm and tobacco budworm (*Heliothis virescens* (F.)) activity appeared greater in the Thimet plots than in the untreated check or toxaphene treatment (table 4). A partial explanation may be in the attractiveness of the luxuriant foliage and delayed maturity associated with Thimet seed-treatments of cotton.

Thimet exhibited a significant advantage in yield at only one location, Union County, experiment 1 (table 5). A heavy thrips infestation was encountered at this location. However, delayed fruiting still played a role in the total yield. When the effects that depressed the yield in the untreated plots were removed by applications of toxaphene, a significant gain in yield above that resulting

Table 4.—Boll weevil and bollworm infestation in field Experiment 3.[a] North Carolina, 1956.

Date	Treatments[b] Check	Toxaphene, 4.0 lbs.	Thimet, 0.5 lb.	Thimet, 1.0 lb.	L.S.D. 5%
		Scotland County			
		Per cent squares punctured by weevils			
June 26	14.0	2.8	5.0	4.2	
July 3	9.5	1.0	0.8	0.2	
9	2.2	1.8	2.5	1.8	
16	28.5	9.5	9.2	2.8	
24	62.0	16.2	41.0	22.5	
30	85.0	28.8	69.8	61.8	
Aug. 8	98.8	63.5	85.5	77.5	
Average	42.9	17.7	30.5	24.4	7.2
		Adult weevils per 100 squares			
Average	6.2	0.8	2.4	1.7	4.2
		Per cent squares injured by bollworms			
June 26	4.8	3.5	10.8	21.0	
July 3	3.8	3.2	12.5	14.2	
9	0 5	0.5	0.5	0.8	
16	1.0	1.8	0.5	0.8	
24	0.0	0.0	1.8	1.0	
30	2.8	1.8	6.0	9.2	
Aug. 8	5.0	3.8	16.5	18.8	
14[c]	7.2	0.5	20.2	25.2	
22[c]	10.5	2.2	12.0	21.2	
Average	4.0	1.9	9.0	12.5	2.8
		Bollworm larvae per 100 squares			
Average	.9	.2	1.2	1.3	.8
		Cleveland County[d]			
		Per cent squares punctured by weevils			
July 2	4.5	0.0	2.5	0.5	
10	5.0	0.5	6.8	1.5	
17	7.5	0.2	1.5	0.8	
23	23.0	9.2	10.2	10.2	
31	44.8	6.5	33.2	21.0	
Aug. 7	40.0	7.0	28.2	19.2	
Average	20.8	3.9	13.7	8.9	6.3
		Adult weevils per 100 squares			
Average	1.2	0.1	0.7	0.1	n.s.
		Per cent squares injured by bollworms			
July 2	0.5	0.0	0.0	0.8	
10	0.0	0.2	0.5	0.0	
17	0.8	2.2	0.2	0.8	
23	1.8	2.8	1.2	1.2	
31	4.0	0.8	3.0	6.0	
Aug. 7	1.8	0.5	1.5	2.0	
13[c]	1.0	0.2	2.2	1.8	
Average	1.4	1.0	1.2	1.8	n.s.

[a] Infestation based upon 100 random examinations per replicate.
[b] Twenty pounds per acre of 20% toxaphene dust at 5-day intervals, pounds per acre of Thimet as seed treatments only.
[c] Per cent injured bolls.
[d] Seasonal average of bollworm larvae very low.

Table 5.—Yield of cotton in field tests for control of cotton insects. North Carolina, 1955–56.

County		Pounds of Seed Cotton per Acre[a] Check	Toxaphene	Thimet, 0.5 lb.	Thimet, 1.0 lb.	L.S.D. 5%
		Experiment 1[b]				
Cleveland	First	896	1137	695	—	136
	Total	1713	1774	1455	—	n.s.
Union	First	887	1099	668	—	288
	Total	1380	1699	1525	—	128
Scotland	First	1106	1118	788	—	n.s.
	Total	1513	1571	1351	—	108
Halifax	Total	941	943	558	—	204
		Experiment 2[b]				
Union	First	1005	1133	854	813	n.s.
	Total	1280	1379	1235	1260	n.s.
Johnston	First	1354	1300	1384	1261	n.s.
	Total	1549	1459	1736	1706	n.s.
		Experiment 3[c]				
Cleveland	First	675	789	542	582	n.s.
	Total	1125	1263	1261	1220	n.s.
Scotland	First	1314	1448	1112	883	288
	Total	1729	1930	1633	1422	n.s.

[a] Yield based upon harvest of one-twentieth acre near center of each replicate.
[b] Early-season experiments for control of thrips.
[c] Experiment on seasonal control of boll weevil and bollworm.

from Thimet treatment was obtained. In experiment 2 a heavy spider mite infestation was encountered in Johnston County. Substantial increases in yield were obtained in the Thimet-treated plots (table 5). Thus, the use of Thimet appeared profitable when a damaging infestation existed which was controlled by Thimet and where the delayed portion of the crop, associated with Thimet treatments, was not damaged by late-season insects or adverse weather conditions.

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Effect of Ionization upon Penetration of Organophosphates to the Nerve Cord of the Cockroach[1]

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Abstract

The penetration *in vitro* into the nerve cord of the American cockroach (*Periplaneta americana* (L.)) has been studied for three organophosphates. TEPP, which is unionized, penetrates about as rapidly into the intact as into the excised cord. "Quaternarized Tetram" (the iodide of $(C_2H_5O)_2P(O)SCH_2CH_2N(C_2H_5)_3$) which is ionized, penetrates the excised cord well, but the intact cord extremely poorly; these effects are independent of pH. Tetram® (R-6199, oxalic acid salt of $(C_2H_5O)_2(PO)SCH_2CH_2\overset{+}{N}(C_2H_5)_2$) an ionizable compound, penetrates the excised cord well and independently of pH, but penetrates the intact cord poorly at low pH (when Tetram is ionized) and well at high pH (when it is unionized). The results confirm the presence of an ion-barrier in the intact cord, and demonstrate the importance of avoiding basicity in insecticidal organophosphates.

There is evidence that several potentially toxic compounds are without effect upon insects because they fail to penetrate the nerve cord (Twarog & Roeder 1957, O'Brien & Spencer 1957, O'Brien 1957, O'Brien & Fisher 1958, Winton *et al.* 1958). In most cases, these compounds are ionized, and therefore it is reasonable to postulate an ion-impermeable barrier around the nerve cord, which may be identical with the potassium-barrier described by Hoyle (1953).

The present paper is an attempt to compare penetration of the nerve cord by ionized, unionized, and ionizable organophosphates. The principle used was based upon the observation that hydroxymethyl schradan was ineffective against the cockroach nerve cord *in situ*, but a good inhibitor of the ChE of excised cords (O'Brien & Spencer 1957). Presumably this difference reflects the protective effect of some barrier that is disrupted by excision. The barrier may be a part of the cord proper, and in the excised cords the cut nerves may be the loci of penetration. Alternatively, the external sheath described by Richards & Schneider (1958) may be the barrier, and would be damaged during excision to allow penetration over the whole surface of the excised cord.

In this paper three inhibitors have been studied: TEPP (diethylphosphoric anhydride), Tetram® (R-6199, oxalic acid salt of $(C_2H_5O)_2P(O)SCH_2CH_2N(C_2H_5)_2$) and "quaternarized Tetram" (the iodide of $(C_2H_5O)_2P(O)SCH_2CH_2\overset{+}{N}(C_2H_5)_3$). The first is unionized, the second is ionized under acid and unionized under basic conditions, the third is always ionized. These compounds have been studied at various pH's against various nerve cord preparations: (a) homogenized; (b) excised; (c) intact, as described below.

The long range objective of studies such as those reported here is to investigate the role of ionization in toxicity, explore its variation with species, and attempt to make useful predictions about the permissible limits of pK in projected new insecticides.

Methods.—The insects used were adult male American cockroaches, *Periplaneta americana* (L.); they were inactivated by chilling briefly in a deep-freezer. Excised nerve cords were prepared by removal under cold Ringer solution of the abdominal and thoracic cord; only those with unbroken connectives were used. The Ringer solution was as previously described (O'Brien 1957); for the inhibitory incubations the pH was adjusted by addition of acid or base.

The inhibitory incubations were carried out in 400-ml. beakers containing 100 ml. of Ringer to which the appropriate concentration of Tetram had been added. The beakers were then shaken (excursion 1.5 inches 120 cycles per min.) for 1 hour. The materials incubated were (a) for the "intact" preparations: six cockroaches whose wings, dorsa, and alimentary tracts had been removed. (b) For the "excised" preparation: six excised cords plus the remainder of the six roaches (lacking wings, dorsa, and alimentary tracts). Six excised cords and remainders were incubated in every experiment without inhibitor, as a control. At pH's above 7.5, the beakers were shaken in CO_2-free air; alkali was added periodically to maintain the pH, which was followed with a glass electrode.

After incubation, the excised cords were washed with three portions of 10^{-3} M acetyl choline in 0.9% NaCl, to remove excess inhibitor and halt further inhibition. The insects which still contained their cords were washed in this solution, immersed in this solution which had been chilled, and the nerve cords dissected out. The six cords were homogenized in 5 ml. of 0.9% NaCl containing 10^{-3} M ACh, using an iced Potter-Elvejhem type glass homogenizer. From this, triplicate 1.5-ml. samples were added to iced Warburg flasks along with 0.25 ml. of 1.25% $NaHCO_3$ and water to a volume of 2.5 ml. After gassing and equilibration as given below the manometers were closed, 0.2 ml. of 1.8% ACh Cl was tipped in, and readings commenced.

In the homogenate studies with nerve cords, groups of eight cords were homogenized in 1.63 ml. of the following buffer: 250 ml. each of 2.43% tris(hydroxymethyl) aminomethane, 3.4% KH_2PO_4 and water, pH adjusted with acid or base, and volume made to 1,000 ml. Then 4.87 ml. of the same buffer was added, the homogenate was mixed and the actual pH measured (this differed from the pH of the buffer used; thus buffer pH 9.5 plus homogenate gave pH 8.9). To each Warburg flask was added 0.5 ml. of the appropriate inhibitor, then 1.5 ml. of the buffered homogenate. Precisely 60 minutes later further inhibition was stopped by adding 0.7 ml. of the following solution: 4 vols. of 2% ACh, 5 vols. of 1.25% $NaHCO_3$ and 5 vols. of water. The flasks were then put on the manometers, which were placed in the bath (25° C.) gassed with 5% CO_2 in N_2 (3 min.), equilibrated for temperature (3

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minutes) then closed and readings commenced. Five readings were taken, at 10-minute intervals, and results used only if linear CO_2 output was observed.

RESULTS.—*Methodology.*—Kewitz & Nachmansohn (1957) and Van Asperen (1958) have pointed out the possible artefacts arising from homogenizing tissues which contain organophosphates: homogenization may bring into contact with ChE some inhibitor which was spatially separated from it before, and thus one may have an erroneously high estimate of the *in vivo* inhibition. In the present study this effect was avoided by homogenizing in buffer containing substrate (Van Asperen 1958). Unfortunately, in this procedure one does not know how much substrate is still present at the time of measuring ChE activity, since some has been consumed by the ChE; the knowledge is necessary since ChE has a clear substate optimum. Preliminary experiments were therefore used to establish empirically if additional ACh should be added just before measuring; it was found to be necessary. The amount of additional ACh varied with the level present in the homogenate. With 6×10^{-4} M ACh in the diluted homogenate, an addition of 0.2 ml. of 1.8% ACh was optimal.

In the case of homogenates, the first ACh addition was required to block further inhibition by the substantial amount of Tetram present, and in this case it was considered best to add all the ACh at once, in order to be assured of adequate protection. Experiments showed that with such a single addition, an ACh concentration of 8.1×10^{-3} M gave optimal results. With both of the above methods, some error is unavoidable: preparations of high ChE content will consume more ACh prior to measurement, and so will be measured at a lower substrate concentration. This problem is minimal in the technique using two ACh additions, when the second addition can be arranged to make the major contribution to ACh concentration; variations in ACh remaining from the first addition are then less important.

The homogenate technique at various pH's as described above, depends upon the fact that the bicarbonate and CO_2 treatments brings all the flasks to a similar pH, even though they are initially buffered at different pH's. Naturally, this is only approximately true (and becomes less true the more strongly they are initially buffered). The technique was resorted to because of pipetting difficulties when an otherwise more desirable procedure was used: at first, concentrated enzyme-inhibitor preparations were incubated, and then samples were diluted down for assay, thus reducing interfering buffer action. However, the incubated preparations seemed to change physically, so that it was hard to duplicate the sampling accurately. Fresh enzyme preparations were easily pipetted with accuracy; hence the device was used of carrying out inhibition and assay in the same Warburg flask. Table 1 shows how the initial pH was changed on addition of the bicarbonate (for the enzyme was exposed under these conditions prior to gassing) and the final pH after gassing. These final pH's which represent the pH's at which the actual ChE measurements were made, vary from 7.05 to 7.30. But as each inhibited sample was compared with an identically treated control, this degree of variation was considered permissible.

Table 1.—**Effect of added bicarbonate and subsequent gassing upon buffer at various pH's.**[a]

INITIAL pH	pH AFTER $NaHCO_3$	pH AFTER $NaHCO_3$ AND GASSING
6.50	7.00	7.05
7.00	7.32	7.15
7.50	7.66	7.20
8.00	7.99	7.21
8.50	8.40	7.30

[a] Concentrations of constituents were as in the Warburg flasks. Buffer: phosphate-tris(hydroxymethyl)aminomethane as in "Methods." Measurements with glass electrode. Final pH "after gassing" taken while passing 5%-CO_2-N_2 through solution.

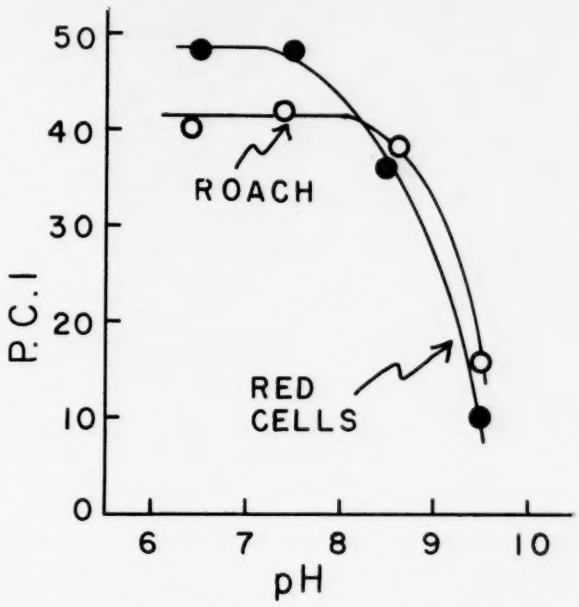

FIG. 1.—The effect of pH upon per cent cholinesterase inhibition (P.C.I.) of cholinesterases of cockroach nerve cord and human red cells. Inhibitor: Tetram pI 7.65, 25° C., 60 min. incubation.

Homogenates.—The inhibition of cockroach cord homogenates by Tetram as a function of pH is shown in figure 1. Results for human erythrocyte cholinesterase are also shown. Both behave in the same way.

The pKa of Tetram was found by titration using a glass electrode, and determining graphically the inflection point of the plot of pH versus volume of titrant. It was found to be 8.4. The results of figure 1 therefore suggest that the free-base form of Tetram is a poorer inhibitor of cholinesterases than the protonated form. However, figure 1 will also reflect variations in the capacity of the ChE to be inhibited, as a result of changes in the ionization of the esteratic site (Wilson & Bergmann 1950).

Homogenized Versus Excised Nerve Cords.—A comparison was made of the effectiveness of the three inhibitors against homogenized as compared with excised cords at a fixed pH. The results (fig. 2) show that: (a) with excised cords, cholinesterase inhibition varies with inhibitor concentration in a regular way, so that inhibition does give an index of the amount of effective inhibitor present. This fact is utilized later in this paper; (b) as expected, the cholinesterase in excised cords is less sensitive than in homogenates. This protective effect is somewhat more

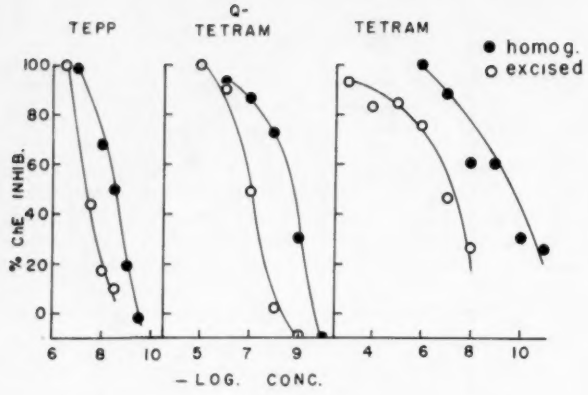

FIG. 2.—Effect of inhibitors upon homogenized and excised nerve cords at fixed pH.

pronounced for Tetram and its quaternary analogue than for TEPP but is of the same order of magnitude for all three compounds. Therefore ionization is not of extreme importance in governing penetration into the excised cord.

Excised Versus Intact Nerve Cords.—A comparison was now made of the inhibitors against excised as compared with intact cords, using a fixed inhibitor level but varying the pH. With TEPP (fig. 3) little difference was seen with either preparation, nor was pH of much importance. Since with soluble preparations, pH has an important effect upon inhibition by TEPP (Wilson & Bergmann 1950) the observation implies that modifying the external pH has little effect upon the pH at the enzyme site. Clearly any barrier present around the intact cord has negligible effect upon TEPP penetration.

With quaternarized Tetram (fig. 4) there was an extreme difference between the intact and excised preparations, and this difference was almost independent of pH. From the results on excised cords in figure 2 one may calculate approximately that the 25% inhibition observed in the intact cords implies that the effective concentration of inhibitor inside the barrier was about 3×10^{-8} M. The external level was 10^{-6} M. Thus only 3% of the external level was achieved internally. The barrier is therefore very effective, yet is not completely exclusive. Furthermore its effectiveness does not vary within the pH range 5–8.

With Tetram (fig. 5), a very clear pH dependence of inhibition is seen with the intact cord, but very little with

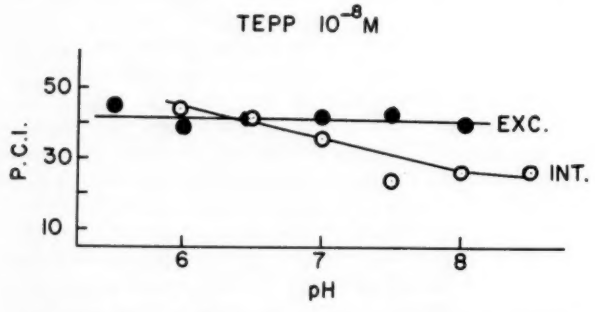

FIG. 3.—Effect of pH upon per cent cholinesterase inhibition (P.C.I.) produced by TEPP in intact and excised nerve cords.

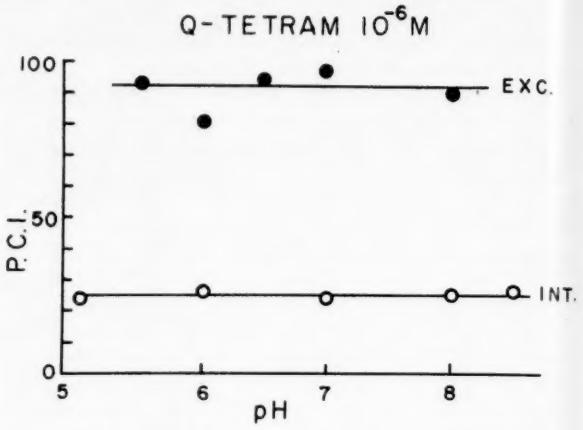

FIG. 4.—Effect of pH upon per cent cholinesterase inhibition (P.C.I.) produced by quaternarized Tetram in intact and excised nerve cords.

the excised. From the data at high pH, it seems that even the unionized form penetrates poorly. Using figure 2 one may calculate that at pH 9 the internal level is about 10% of the external. But at low pH, the effect is much more marked: at pH 5.5 the internal level is about 10^{-8} M, or 0.3% of the external. These calculations are sufficiently crude that this figure of 0.3% may perhaps not be significantly different from the 3% figure reported for the quaternary analogue. For example, in figure 5, 3×10^{-6} M Tetram gave about 95% inhibition of excised cord enzyme, whereas in figure 2, this level gave about 80%.

The data so far reported suggest qualitatively that the ionized form of Tetram (at low pH) penetrates less readily than the unionized form (at high pH). The quantitative aspects are less satisfactory. The pH of Tetram is 8.4. Thus between pH 7 and 9 there is maximal variation in

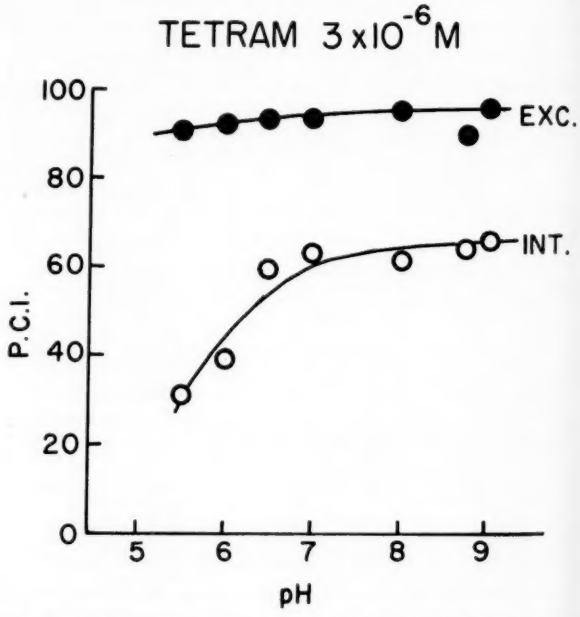

FIG. 5.—Effect of pH upon per cent cholinesterase inhibition (P.C.I.) produced by Tetram in intact and excised nerve cords.

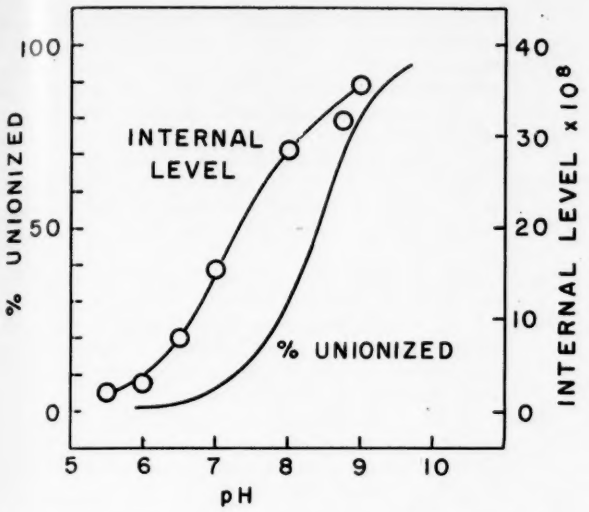

FIG. 6.—Variation with pH of per cent of Tetram which is unionized, and of the molar level of Tetram inside the intact nerve cord (calculated from data of figure 5).

the extent of ionization, and between 5.5 and 7 this variation is slight. Yet figure 5 shows that variation in inhibition is maximal between 5.5 and 7, and small between 7 and 9.

Figure 6 shows (a) a graph calculated from a pKa of 8.4 showing the per cent of Tetram unionized at any given pH. (b) A graph calculated from the data of figure 5 (on the basis of results in figure 4 for Tetram versus excised cords) showing the variation in internal level of Tetram as a function of pH. The calculations are made from points read off the smooth curve, not from the actual experimental points. By "internal level" is meant the concentration within the hypothetical barrier.

The results are less disturbing when transformed in this way, but still unsatisfactory, particularly in the variation of internal level between pH 5.5 and 6.5, which is unaccompanied by any important variation in the percentage of ionization. If one subtracts the numerical values of the "per cent ionization" curve from those of the "internal level" curve of figure 5, a curve is obtained showing a distinct optimum at pH 7.5. This qualitative picture of the interfering factor shows that at pH 7.5 there is maximal departure from prediction.

Toxicity.—Table 2 shows LD_{50}'s (by injection) to the cockroach, house fly, (*Musca domestica* L.), mouse, and rabbit. It is clear that Tetram is much less toxic to the insect than to the mammal. The quaternary analogue is more toxic than Tetram to the mouse, but much less toxic to the insect. These striking data are not attributable to a low activity against insect cholinesterase, as shown by the pI 50 values given in table 2. Since the compounds were injected, the factor of integumental penetration has been avoided. It seems clear that ionization greatly reduces toxicity to the insect, but not to the mouse.

DISCUSSION.—The results of figure 1 shows for the first time that cockroach cord cholinesterase has an anionic site, precisely as mammalian true cholinesterase.

In an earlier work (O'Brien & Fisher 1958) it was felt to be a "disturbing anomaly" that Tetram was appreciably toxic to insects, since it was then believed that its pKa would be about 10.8 (Spencer & O'Brien 1957) and it would be effectively fully ionized in the insect. However, studies by Tammelin (1957) on related compounds suggested a pKa of about 8 for Tetram, and actual measurement gave a figure of 8.4. It was therefore apparent that at least a small percentage would be unionized at physiological pH, and that this might account for the limited insecticidal action.

Table 2.—Toxicity and anticholinesterase activity of Tetram and quaternarized Tetram.

		TETRAM	QUATERNARIZED TETRAM
LD_{50} (γ/g.)	House fly	ca. 1000[a]	>1000[b]
	Cockroach	93	400
	Mouse female intraperitoneal	0.42	0.17
	Rabbit[c] (intravenous)	0.3	0.085
pI_{50}	Cockroach nerve cord	8.7	8.8
	House fly head	9.2	10.65
	Rat brain	10.7	10.6
	Human erythrocyte	8.9	9.5
	Human plasma	10.3	9.3

pI_{50}: negative log of molar concentration to inhibit enzyme 50%. (determined at 25° C., 90 min. incubation, pH 7.2 with homogenates).
[a] 40–50% kill at 500, 750 and 1000 γ/gm.
[b] 20% kill at 1000 γ/gm., 0% at 750 γ/gm. and below.
[c] Date of Koelle (1957).

The results in this paper show that Tetram has an extremely low toxicity to insects, in spite of being an excellent inhibitor of insect cholinesterase *in vitro;* and that this low toxicity can be reliably attributed to its poor penetration of the nerve cord. Its quaternary analogue is even less toxic (although an even better anti-cholinesterase) due to its still poorer penetration power. Penetration appears to be governed by charge, so that the unionized species of Tetram penetrates more readily than the ionized.

Boon (1957) claimed good control of eight mites and eight scales by Tetram—but a 400 p.p.m. solution was needed for scales. Pea aphids (*Macrosiphum pisi* (Harr.)) are also susceptible (Ghosh & Newman 1955). Presumably these arthropods do not have an efficient ion-barrier, or else some other target than nerve-cord cholinesterase is attacked. The former hypothesis has been examined (O'Brien & Fisher 1958) but no evidence obtained for it.

We may conclude from this and a preceding study (O'Brien & Fisher 1958) that the introduction of an ionized or readily ionizable group into an anticholinesterase will make the compound a selective mammalicide. However, some arthropods (mites, aphids) may be susceptible. If other pests than these are to be poisoned, it is desirable to avoid ionizable compounds, unless the pKa is low in the case of bases (*e.g.*, below 7) or high in the case of acids (*e.g.*, above 7).

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Insecticidal Control of Insects Infesting Broccoli and Cabbage[1]

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ABSTRACT

Numerous insecticides were tested for effectiveness of control of the cabbage looper, *Trichoplusia ni* (Hbn.); the imported cabbageworm, *Pieris rapae* (L.); and the cabbage aphid, *Brevicoryne brassicae* (L.), on broccoli and cabbage. The insecticides were applied as sprays, either alone or in combination, at weekly intervals. The most promising materials against all three pests were dimethoate and malathion (2 qt.) alone or in combination with Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane) or toxaphene. Perthane-Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dibrom® (*O,O*-Dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)-Perthane also gave good protection to broccoli. Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl)phosphorodithioate), Delnav® (2,3-*p*-dioxanedithiol-*S,S*-bis (*O,O*-diethyl phosphorodithioate), Kepone® (1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo (5.2.1.0^{2,6}.0^{3,9},0^{5,8}) decan-4-one) and Kepone-malathion did not control aphid satisfactorily. Am. Cyanamid 18706 (*O,O*-dimethyl *S*-(N-ethylcarbamoylmethyl) phosphorodithioate), Delnav, phosphamidon, and malathion (1 qt.) were not effective against the cabbage looper. All insecticides showed some burning of broccoli in mid-September. However, all traces of phytotoxicity disappeared by harvest time.

Observations were made on the effectiveness and possible phytotoxic effects of several insecticides and combinations thereof, some at twice the dosage normally recommended, against insects attacking broccoli and cabbage. The insects against which control was primarily directed were the cabbage looper, *Trichoplusia ni* (Hbn.); the imported cabbageworm, *Pieris rapae* (L.); and the cabbage aphid, *Brevicoryne brassicae* (L.).

MATERIALS AND METHODS.—Broccoli was transplanted on July 31, 1958, into 64 two-row plots, each row 30 feet long, with 3 feet between rows. Plots were separated by a 6-foot gap. Sixteen treatments, including 2 checks, were randomized in 4 replicates. The insecticides used are listed in table 1 with the rate of actual material applied per acre at each treatment date. Insecticides were applied as a spray, using the desired amount of emulsifiable concentrate in 25 gallons of water per acre on the first treatment date, and in 50 gallons on all succeeding treatment dates. Sprays were applied with an improved boom (Burkhardt & Ditman 1956) using five nozzles per row at 150 p.s.i. pressure. All plots were treated on August 12, 19, 26, September 2, 9, 16, 24, October 2, 10, and 17.

On September 15, all plants were examined for presence of insects and for any possible damage that might be attributable to the phytotoxic nature of the insecticides. On the same day the number of plants infested with the cabbage looper and/or the cabbageworm was recorded (table 1). At this observation date, no heads were formed on broccoli and no aphids were observed. Broccoli heads were harvested weekly from September 30 to October 28. Infestation of harvested heads is recorded in table 2. On October 8, plants were rated as to the extent of foliar damage and classified into three categories, clean, light injury, and heavy injury (table 2). Head samples were taken from three replicates on October 7 for flavor evaluation. Floret samples were taken from all replicates for flavor evaluation on October 30.

Cabbage was transplanted on July 25 into 28 two-row plots with the same specifications as the broccoli plots.

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Table 1.—Insect infestation records on broccoli (September 15, 1958).

Treatment	Quarts/A.	Pounds Actual per Gal.	Per Cent of Plants Infested with		Total Infested (%)
			Cabbage Looper	Cabbage Worm	
1. Perthane	1	4	0	0	0
+malathion	1	4			
2. Perthane	2	4	0	0	0
+malathion	2	4			
3. Malathion	2	4	0	0	0
4. Toxaphene	2	6	0	0	0
+malathion	2	4			
5. Perthane	1	4	0	0	0
+Diazinon	1	2			
6. Dibrom	1	4	0	0	0
7. Check	—	—	51.0	34.0	75.5
8. Thiodan	1	2	0	0	0
9. Dimethoate	1	4	0	0	0
10. Am. Cyanamid 18706	1	2	31.5	3.8	33.8
11. Trithion	1	4	1.5	0	1.5
+Perthane	1	4			
12. Guthion	1	1.5	1.5	0	1.5
13. Delnav	1	4	30.2	0	30.2
14. Check	—	—	46.5	30.2	63.6
15. Kepone	2	2	0	0	0
16. Kepone	2	2	0	0	0
+malathion	1	4			

Seven treatments, including a check, were randomized in four replicates. With the same equipment as used for broccoli the first three applications of all insecticides (table 3) were made in 25 gallons of water per acre, succeeding applications in 50 gallons of water per acre. Treatment number 7 was applied for the first two applications, and then not at application number 3, but again on all following dates. Cabbage plots were sprayed on July 29, August 5, 12, 19, 27, September 3, and 10.

On August 26, infestation records were taken from all plots. The number of plants infested with cabbage looper and/or imported cabbageworm was recorded (table 3). No aphids were present at this time. Cabbage was harvested on September 17 and 26. The heads were rated for injury and a record of the number of cabbage loopers and cabbageworms found was taken (table 4). Only two plants infested with aphids were observed at harvest, both in the check plots, and are therefore not listed in the tables.

DISCUSSION OF RESULTS.—Table 1 shows that all but two treatments used gave excellent control of both cabbage looper and cabbageworm on broccoli. Am. Cyanamid 18706 (*O,O*-dimethyl *S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate) and Delnav® (2,3-p-dioxanedithiol *S*,bis*S*(*O,O*-diethyl phosphorodithioate)) were distinctly inferior to all other treatments in the control of the cabbage looper. As recorded in table 2, severe foliage feeding in plots treated with these two insecticides was caused primarily by cabbage looper. All other treatments show excellent reduction in foliar injury in comparison with the check plots. As to insects in the heads at harvest, all treatments were effective against the cabbage looper and the cabbageworm. Guthion® (*O,O*-dimethyl-*S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl)phosphorodithioate) Delnav, Kepone® (1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one) and Kepone-malathion were not much better than the checks in degree of aphid infestation. All other treatments considerably reduced aphid populations. However, only dimethoate (*O,O*-dimethyl *S*-(N-methylcarbamoylmethyl) phosphorodithioate) and Am. Cyanamid 18706 produced heads completely free of aphids. All treatments showed evidence of insecticidal burning on September 15. The Kepone-malathion and Delnav treatments showed the most serious burning on leaves. Malathion at the 2-quart rate showed somewhat less burning, but still more than the remainder of the treatments. Plots treated with dimethoate and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) appeared most vigorous at this time. With cooler and more moist weather during the latter half of September, all evidence of burning disappeared, and no phytotoxic effects from any treatments were apparent by September 30.

All treatments that included malathion gave reasonably good aphid control, except malathion in combination with Kepone. This might possibly indicate some incompatibility between these two insecticides. However, the treatment completely controlled both loopers and cabbageworms.

Yield records on the broccoli plots showed no differences between the treatments. Flavor evaluation tests showed no significant differences between the treatments. No insecticidal off-flavors were detected.

On cabbage, the results in table 2 show that Am. Cyanamid 12880 (treatment No. 1) gave the best control of cabbage looper and imported cabbageworm. Malathion at the 2-quart rate was less effective against cabbageworm than it was against the looper. However, both these treatments gave considerable reduction in infestation in comparison with the check. Dimethoate (treatment No. 7) was poorer than treatment No. 1, possibly because of the omission of one application 2 weeks prior to these counts. In table 4 both treatments with dimethoate gave the highest percentage of clean heads and the least number of cabbage looper per head. Malathion at the 2-quart rate was next in effectiveness, still considerably better than the check. All treatments gave effective control of the cabbageworm. No evidence was given of insecticidal phytotoxicity of any of the treatments on cabbage at any time during the experiment. Cabbage yields were quite similar in all treatments and checks and are therefore not listed.

In viewing the results on both broccoli and cabbage, it is immediately apparent that dimethoate shows great promise in the control of the cabbage looper, imported cabbageworm, and cabbage aphid. Next in effectiveness against all three pests are the treatments utilizing malathion at the 2-quart rate either alone or in combination with Perthane® [1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane] or toxaphene. Malathion at this rate shows a tendency to burn broccoli, but under conditions prevalent during the 1958 season no serious phytotoxic effect resulted. Malathion at the 1-quart rate gave good results against all three pests when in combination with Perthane, but with Kepone its effectiveness was greatly reduced against the cabbage aphid. Malathion at 1 quart

Table 2.—Plant injury (October 8, 1958) and degree of infestation of broccoli heads at harvest (September 30–October 28, 1958).

Treatment	Quarts/A.	Per Cent of Plants with Injury			Per Cent of Heads with			
		None	Light	Heavy	None	Looper	Worm	Aphid
1. Perthane +malathion	1 1	94.2	4.1	1.7	96.2	0	0	3.9
2. Perthane +malathion	2 2	98.6	1.5	0.0	93.8	0	0	6.3
3. Malathion	2	94.2	3.9	2.0	95.2	0	0	4.8
4. Toxaphene +malathion	2 2	98.5	1.5	0.0	93.5	0	0	6.5
5. Perthane +Diazinon	1 1	88.4	5.8	5.8	95.4	0	0	4.6
6. Dibrom	1	90.6	7.9	1.6	97.3	0.9	0	1.8
7. Check		21.1	0.6	78.2	61.4	6.1	19.7	16.7
8. Thiodan	1	94.2	4.2	1.7	93.8	0.8	0	5.4
9. Dimethoate	1	93.1	6.2	0.8	100.0	0	0	0
10. Am. Cyanamid 18706	1	53.2	16.7	30.2	100.0	0	0	0
11. Trithion +Perthane	1 1	92.8	3.6	3.6	95.5	0	0	4.5
12. Guthion	1	90.9	2.3	6.8	89.9	0	0	19.0
13. Delnav	1	64.2	10.2	25.6	87.0	0	0	13.0
14. Check		2.3	15.6	82.0	61.2	6.0	13.8	20.7
15. Kepone	2	94.4	4.9	0.7	80.3	0	0	19.7
16. Kepone +malathion	2 1	100.0	0.0	0.0	88.0	0	0	17.0

Table 3.—Insect infestation records on cabbage (August 26, 1958).

Treatment	Quarts /A.	Pounds Actual /Gal.	Per Cent Plants with		Total Infested[a] (%)
			Looper[a]	Worm[a]	
1. Dimethoate	1	4	1.55	4.65	5.43
2. Phosphamidon	1	4	28.06	4.32	32.57
3. Malathion	1	4	11.57	13.22	24.79
4. Check	—	—	31.70	34.40	60.80
5. Malathion	2	4	4.65	9.30	13.95
6. Am. Cyanamid 18706	1	2	26.27	26.27	49.15
7. Dimethoate[b]	1	4	16.03	12.98	29.01

[a] Counts made 1 week after application (2 weeks in case of Treatment 7).
[b] Applied for first 2 weeks—then skipped 1 week and applied every week thereafter.

Table 4.—Plant injury and degree of infestation of cabbage at harvest (September 17–26, 1958).

Treatment	Quarts/ A.	Per Cent Heads with Injury			No. per Head	
		None	Light	Heavy	Loopers	Worms
1. Dimethoate	1	80.3	19.7	0.0	0.06	0.0
2. Phosphamidon	1	32.6	63.0	4.4	.57	.05
3. Malathion	1	49.1	50.0	0.9	.31	.01
4. Check	—	0.0	32.2	67.8	.95	1.27
5. Malathion	2	67.5	31.7	0.8	.13	.01
6. Am. Cyanamid 18706	1	0.9	67.9	31.3	.37	.0
7. Dimethoate	1	83.2	16.8	0.0	.03	.02

did not give satisfactory looper control on cabbage. Perthane-Diazinon®, (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate) Trithion® [*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate]-Perthane, Dibrom® [*O,O*-Dimethyl-O-(1,2-dibromo-2,2-dichloroethyl) phosphate), and Thiodan gave good protection on broccoli. Am. Cyanamid 18706 was excellent against aphid and cabbageworm, but ineffective against looper at the concentration used. Guthion and Kepone were satisfactory against looper and cabbageworm, but ineffective against cabbage aphid. Delnav gave good control of only the cabbageworm and was greatly inferior in controlling looper and aphid. Phosphamidon was also ineffective against the cabbage looper.

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Arthropod Fauna Found During the First-Season Trial of a Selective Spray Schedule in a New Jersey Apple Orchard[1]

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Abstract

The insect and mite populations in two neighboring blocks of apple trees in a southern New Jersey orchard were studied to determine the nature of the populations where a ryania-glyodin spray program was used to eliminate certain complications associated with chlorinated hydrocarbon insecticides.

Records of spider mite numbers and collections of predaceous arthropods were taken at weekly intervals. At the end of the season injury to the fruit from codling moth (*Carpocapsa pomonella* (L.)) and plum curculio (*Conotrachelus nenuphar* (Hbst.)) was determined.

The data indicated that a glyodin-ryania schedule can provide acceptable codling-moth control together with satisfactory control of phytophagous mites.

The insecticide ryania when used in protection sprays for apples has been shown to provide satisfactory control of the codling moth, *Carpocapsa pomonella* (L.), without serious outbreaks of phytophagous mites (Hamilton & Cleveland 1957). Ryania sprays containing the fungicide glyodin appear to suppress phytophagous mite populations (Clancy & McAlister 1956). Ryania-glyodin spray combinations are thought to be sufficiently selective that certain natural enemies of the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), are not eliminated. This supposition is in contrast to the studies which have shown that many spray chemicals, particularly DDT, are detrimental to important predator species. (Pickett & Patterson 1953, Clancy & Pollard 1952.)

The following information has been obtained from a study of the fauna in a southern New Jersey apple orchard where a ryania-glyodin spray program was followed during the growing season of 1957. The 30-year-old trees used for testing were Stayman and McIntosh varieties, situated in a 1-acre block (block A) and a nearby 8-acre block (block B). Block B was bordered by woods on one end, by a highway on the other end, and by tomato and corn fields on the sides. Block A was surrounded by a woodlot on three sides and by a tomato field on the remaining side.

Spray Schedule.—All sprays were applied by the grower with a John Bean speed-sprayer. Glyodin was applied to block A throughout the season and in block B until the second spray following the petal-fall spray, May 24, at which time captan was applied and continued in three subsequent sprays. Glyodin was applied in this block again in the last two sprays in July.

Demeton, ½ pint per 100 gallons, was used in the pink-stage spray in both blocks. Both blocks received lead arsenate, 2½ pounds per 100 gallons, in the petal-fall-stage spray and the two following sprays at 10-day intervals. Ryania, 5 pounds per 100 gallons, was applied to both blocks beginning with the last spray containing lead arsenate and continuing at 10-day intervals in all the cover sprays.

Sampling Procedure.—At weekly intervals, 20 leaves were collected from each of five trees in each end of the blocks; 10 leaves from foliage around the inside of the skirt of the tree; and 10 leaves from the outside of the tree. The leaves were taken to the laboratory and the mite populations were determined with a leaf-brushing machine (Henderson & McBurnie 1943).

Table 1.—Arthropods found in Blocks A and B on Staymen and McIntosh receiving ryania schedule.

	Mites and Eggs per Leaf						Predaceous Insects per Tray	
	Panonychus ulmi		*Mediolata* sp.		*Typhlodromus* sp.			
	A	B	A	B	A	B	A	B
May 29	0	0	0	0	0	0	14	1
June 27	1	3	0.1	0	0	0	5	—
July 4	13	21	1.0	0.4	0.1	0	10	5
July 11	30	48	1.0	0.2	0	0.2	56	16
July 18	51	31	2.5	0	0.1	0.4	124	43
July 25	55	34	4.0	0	0.1	0.1	193	86
Aug. 1	53	49	8.7	0	0.1	0.3	175	101
Aug. 6	40	61	7.2	0.5	0.2	0.2	235	172
Aug. 9	38	47	6.5	0.5	0.2	0.2	—	—
Aug. 15	7	14	5.1	0.6	0.1	0.3	34	186
Aug. 20	7	13	3.3	0.2	0.1	0.6	10	71
Aug. 24	11	12	5.7	0.5	0.2	0.3	7	108
Aug. 29	7	12	7.9	1.4	0.1	0.3	8	49
Sept. 3	5	7	10.5	1.1	0.1	0.4	6	25
Sept. 10	6	16	13.0	1.6	0	0.5	4	27
Totals	324	368	76.5	7.0	1.4	3.8	881	890

The predator populations in the experimental blocks were also sampled on a weekly basis. The principal predacious arthropods, which were noted and recorded in weekly collections, were: mites of the genera *Typhlodromus* and *Mediolata*, the coccinellid *Stethorus punctum* (Lec.), the anthocorid *Orius insidiosus* (Say), *Haplothrips faurei* (Hood), *Leptothrips mali* (Fitch), and *Chrysopa aurata*. In addition, the mirids *Hyaloides harti* (Knight) and *Phytocorus* sp. were noted occasionally, although these were encountered in very minor numbers.

Samples for estimating the predator population were collected according to the technique developed by Pickett and his coworkers. This method consists of tapping with a light stick a number of peripheral limbs of the trees over a tray of standard size and removing the fallen arthropods by an aspirator (Lord 1949). The specimens were removed in vials of alcohol for examination in the laboratory. The predatory mites were collected at the time of leaf examination for phytophagous mites.

Table 1 shows a comparison of the arthropods found on the two blocks of trees during the summer of 1957.

Results.—European red mites were the only phytophagous mites found in appreciable numbers. Two-spotted spider mites were found occasionally, but their numbers did not increase in the orchards during the season, although they became quite numerous on the corn and tomatoes surrounding the orchards. The numbers of

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Table 2.—Important insect predators of European red mite recorded from trees on glyodin-ryania schedule in 1957.

	Dates of Collection											
	June	July				August						September	
	25	4	11	18	25	1	6	15	20	24	29	3	10
						Block A							
Thrips spp.	0	2	10	6	33	36	26	6	2	1	0	2	0
Stethorus punctum	0	1	17	61	109	81	58	10	2	0	2	1	2
Chrysopa aurata	2	2	6	8	29	39	128	7	4	1	9	1	1
Orius insidiosus	0	2	16	46	11	2	8	0	1	1	0	1	0
Totals	2	7	49	121	182	158	220	23	9	3	11	5	3
						Block B							
Thrips spp.	0	1	0	9	12	17	35	17	6	6	17	8	2
Stethorus punctum	0	0	4	13	37	38	90	135	50	85	22	11	21
Chrysopa aurata	0	1	4	8	18	37	39	22	13	15	7	1	1
Orius insidiosus	0	2	4	7	1	3	3	1	0	0	0	1	1
Totals	0	4	12	37	68	95	167	175	69	106	46	21	25

European red mites showed some early spasmodic increases in localized areas, apparently because of differences in the initial mortality from demeton. The increase and population peak of European red mites occurred on the trees in block A before the increase and peak on the trees in block B. None of the McIntosh trees developed mite-bronzing, but a few Stayman trees became slightly to moderately bronzed in both blocks. The first European red mite winter eggs were found on August 29.

The predatory mites *Mediolata* spp. and *Typhlodromus* spp. were scarce throughout the early and middle part of the season and apparently contributed very little to the control of the phytophagus mites during that time (table 1). Their populations increased toward the end of the season when they became the principal agents of red-mite control.

Table 2 shows the numbers of the major insect predators found during the season. Among the predatory insects, *Chrysopa aurata* and *Stethorus punctum* were important contributing factors in bringing the phytophagous mites under control (table 2) when the mites were present in "outbreak" numbers. Populations of predatory insects did not begin to increase until after European red mites had built up to a number greater than 10 per leaf. The initial rapid increase of the insect predators was due partially to migrations to the trees from the surrounding environment.

On the basis of hand examination of the fruit prior to harvest, codling moth had injured 1.8% of block A fruit and 2.8% of block B fruit in the 8-acre block. Injury was limited to small pin-point stings.

More apples were injured by the plum curculio, *Conotrachelus nenuphar* (Hbst.), than by all other insects in both orchards, injury being greatest in block A, which was surrounded on three sides by woods. Curculio-injured fruit amounted to 4% in block B, 9% in block A. The red-banded leaf roller, *Agyrotaenia velutinana* (Wlkr.), caused an equal amount of injury to the fruit in both blocks, amounting to slightly over 2%.

The number of apples injured by fruit worms and scab was negligible, and very little foliage injury was found attributable to leaf-feeding insects.

From the above data, indications are that a glyodin-ryania schedule can provide acceptable codling-moth control on apples accompanied by satisfactory control of phytophagous mites. The observations show that a number of predaceous arthropods known to reduce mite populations appear within one season following the discontinuation of DDT sprays.

In contrast to the findings of Clancy & Pollard (1952), *Typhlodromus* spp. mites were not found by the present authors to be notably effective predators on large populations of European red mites. For the first year following DDT application at least, their effect on the phytophagous mite population appeared negligible.

Two commercial blocks of apple orchards were sprayed with chemicals known to be selective in their insecticidal activity. No further acaricides were required following a pink-stage spray of demeton, owing to the action of various biological control agents. Most of the fruit- and foliage-feeding insects were controlled with sprays of lead arsenate and ryania. Severe infestations of plum curculio were not adequately controlled with the chemicals used in these experiments.

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Control of the Sugarcane Borer with Insecticides[1]

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Abstract

In tests conducted in Louisiana in 1956, 1957, and 1958, good control of the sugarcane borer, *Diatraea saccharalis* (F.), was obtained from four biweekly applications of 2% endrin on 30–60 mesh RVM-AA attapulgite granules broadcast over cane fields at the approximate rate of 12 pounds per acre per application. Dust formulations of endrin were not always effective. There was no appreciable difference between the control obtained or yield increases resulting from eight weekly or four biweekly applications of endrin granules.

While some insect control and yield response usually followed treatment with 40% ryania dust, this insecticide was inferior to endrin for controlling the sugarcane borer. An experimental granulated formulation containing 40% ryania gave no better control than the commercial preparation of 40% ryania dust.

A granulated Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) formulation was the only insecticide among several tested which gave borer control comparable to that resulting from endrin treatments.

Treatment with DDT resulted in increased losses caused by the sugarcane borer.

The sugarcane borer, *Diatraea saccharalis* (F.), has been the most serious and destructive insect attacking sugarcane in Louisiana for many years. Present estimates indicate that cane growers of the State have suffered an average annual loss to the sugarcane borer amounting to about 13% of their sugar yields since 1937.

Sodium fluosilicate, cryolite, and ryania dusts have been recommended and used successively for cane borer control in Louisiana since the early 1920's. A dust containing 40% ryania applied at weekly intervals was used almost exclusively by growers for control of the borer for several years prior to 1958.

A summary of results from more than 20 field tests conducted by Dugas *et al.* (1947–1955) indicates that four weekly applications of ryania dust for control of second generation borers gave 48 ± 15% (95% confidence interval) control based upon the percentage of joints bored at harvest. This degree of control gave an average yield increase of 1018 ± 686 pounds of sugar per acre. In other tests eight weekly applications of ryania dust for control of both second and third generation borers showed 68 ± 11% control with an average yield increase of 1433 ± 606 pounds of sugar per acre.

Dugas *et al.* (1952) were first to try endrin for sugarcane borer control in Louisiana. In 1951 they obtained 49% control of sugarcane borers in a field test using an experimental formulation of endrin dust as compared with 97% control from comparable ryania treatments.

In 1955 Dugas *et al.* (1956) showed 50 and 56% control of borers at harvest from four and eight applications of 2% endrin dust, respectively. They found accompanying yield increases of 1004 and 1674 pounds of sugar per acre, respectively. In the same test four and eight applications of 40% ryania dust gave 23 and 45% control with estimated yield increases of 403 and 1,181 pounds of sugar per acre, respectively. In spite of these results they concluded that "there is no place for organics in the cane area as a whole, because of their toxicity to the natural enemies of the borer."

In the presence of accumulated data which indicated that some borer control accompanied by surprisingly large increases in crop yields could be obtained by using from four to eight applications of 40% ryania dust, most growers did not take full advantage of recommended chemical control practices. Those who did use ryania or cryolite usually made four weekly insecticide applications for control of second generation borers, and often supplemented these insecticide applications with releases of *Trichogramma* egg parasites.

Endrin was first recommended for control of the sugarcane borer in Louisiana in 1958 (Long *et al.*, 1958). Field tests conducted since 1955 with endrin and other insecticides are considered in this paper.

Methods of Procedure.—Insecticides were applied by airplane to large-plot tests since aerial application is the most common and often the only practical or possible means of applying chemicals to large acreages of sugarcane. In a small-plot test insecticides were applied from a quart-size glass fruit jar equipped with a metal top in which several holes were punched. The jar was inverted and fastened at an angle to one end of a long pole so that granulated insecticides could be sprinkled over the row from above the tops of tall cane while the worker walked in the middle between rows.

Attempts were always made to apply all of the insecticide formulations used at the uniform rate of 12 pounds per acre per application. The actual rate of application on a few large plots differed appreciably from that intended.

Airplane-treated plots ranged from 1.6 to more than 10 acres, their size and shape commonly being determined by the size, shape, and number of cuts comprising the field to be treated. In the small-plot test, plots were three rows wide and 24 feet long (1/100 acre).

The direct effects of treatments upon borer populations were estimated by determining the percentage of joints or internodes having external signs of borer tunneling at the time of harvest.

Yields from large plots were estimated by weighing machine-cut cane from 300 row-feet, or by hand-cutting and weighing several 1/1000-acre samples from each plot. Weights were always taken from areas approximately equidistant from the sides of the plots and not closer than 50 feet from the nearest headland. Weights were obtained in small plots by hand cutting and weighing all of the cane in each plot.

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[2] Louisiana Agricultural Experiment Station, Baton Rouge, La.

[3] Agricultural Research Service, U. S. Department of Agriculture.

Table 1.—Summary of results from large-plot insecticide experiment conducted on the Bubenzer Farm near Bunkie, Louisiana, during 1956.[a]

Treatments[b]	Per Cent Joints Bored	Yield Increase per Acre	
		Tons of Cane	Pounds of Sugar
Ryania 40% dust	20	2.4	1085
Toxaphene 20% dust	14	4.4	998
Endrin 2% dust	2	6.6	1847
Endrin 2% granules	1	6.7	2050
Untreated	64	21.7[c]	2429[c]
Tukey's D[d]	22	7.2	1179

[a] Sugarcane varieties C.P.36-13 and C.P.36-105, plant cane. Figures are averages of 4 replications.
[b] Eight applications of each insecticide were made at weekly intervals.
[c] Actual yields for untreated plots.
[d] Differences required for significance at the 5% level.

Juice analyses for sucrose and brix content were made on the juice from 10 to 30 stalks selected at random from each plot.

A complete block experimental design was employed in all of these field experiments with treatments assigned at random to each block.

RESULTS.—*1956 Test.*—Eight applications of each insecticide listed in table 1 were made by airplane at weekly intervals to large plots of sugarcane located near Bunkie, Louisiana.

Bored joint counts made at harvest show that endrin dust or granules gave almost complete control. Toxaphene and ryania treatments gave 78 and 69% control, respectively. The difference between each treatment and the check was greater than that required for significance at the 5% level.

Treatment with endrin dust or granules resulted in yield increases of more than 6 tons of cane and approximately 1 ton of sugar per acre. The differences in yields of sugar per acre between plots treated with endrin and plots which received no insecticide treatment were greater than the amount required for significance at the 5% level.

Table 2.—Summary of results from large-plot insecticide experiment conducted on the Island Planting Company Farm near Bunkie, Louisiana, during 1957.[a]

Treatments[b]	Per Cent Joints Bored	Yield Increase or Decrease per Acre	
		Tons of Cane	Pounds of Sugar
Endrin (4) 2% dust	43	− 1.8	− 451
Endrin (8) 2% dust	34	2.4	− 40
Endrin (4) 2% granules	5	8.4	1446
Endrin (8) 2% granules	2	8.0	1450
Toxaphene (4) 20% dust	34	1.3	504
Toxaphene (8) 20% dust	20	3.8	927
Toxaphene (4) 20% granules	17	3.4	1190
Toxaphene (8) 20% granules	13	0.6	392
Untreated	52	27.5[c]	4270[c]
Tukey's D[d]	22	4.9	1860

[a] Sugarcane variety C.P.36-13, plant and second ratoon crops. Figures are averages of 4 replications.
[b] Parentheses contain the number of insecticide applications made (8 weekly or 4 biweekly).
[c] Actual yields from untreated plots.
[d] Differences required for significance at the 5% level.

Yield increases following treatment with toxaphene or ryania were about half as large as those found in endrin-treated plots.

1957 Test.—Endrin and toxaphene were applied by airplane to large plots of sugarcane grown by the Island Planting Company near Bunkie, Louisiana. Table 2 gives the results from this test in which eight weekly applications of each insecticide were made for control of both second and third generation borers.

Endrin granules gave the best control obtained in this test. Results with toxaphene were generally intermediate between those for endrin granules on the one hand and endrin dust on the other. Insecticide granules always gave better control than comparable dust formulations. Eight weekly insecticide applications were generally superior to four biweekly applications, but the difference between four and eight applications was relatively small when granular formulations were applied. An analysis of variance of the bored joint data showed that the average differences between weekly and biweekly insecticide applications, and between dust and granulated formulations were significant at the 1% level. The interaction between insecticides and formulations (carriers) was also significant at the 1% level.

The largest yields of cane and sugar per acre were found in plots receiving four or eight applications of endrin granules. These treatments increased yields by approximately 8 tons of cane and $\frac{3}{4}$ ton of sugar per acre. Applications of endrin dust gave no important yield increases. Plots treated with toxaphene yielded more cane and sugar per acre than check plots, but less cane and sugar per acre than plots treated with endrin granules. An analysis of variance showed that the average difference in yield of sugar per acre between dust- and granule-treated plots was significant at the 1% level. The interaction between insecticides and formulations (carriers) was also significant at the 1% level.

1958 Demonstration Plots.—Table 3 is a summary of results from large demonstration plots located on seven different plantations in six different parishes of southern Louisiana. Four biweekly applications of endrin and ryania for control of second and third generation borers were compared with no treatment. An experimental gran-

Table 3.—Summary of results from large demonstration plots located in several parishes of southern Louisiana during 1958.[a]

Treatments[b]	Per Cent Joints Bored	Yield Increase per Acre	
		Tons of Cane	Pounds of Sugar
Endrin 2% granules	7	7.4	1194
Ryania[c] dust and granules	32	3.3	665
Untreated	37	25.4[d]	4757[d]
Tukey's D[e]	11	3.9	822

[a] Figures are averages of 7 replications located on 7 different plantations in 6 different parishes of southern Louisiana. The sugarcane variety C.P.44-101, C.P.48-103 or C.P.36-13 was grown in all demonstration plots on each plantation.
[b] Four applications of each insecticide were made at biweekly intervals.
[c] Forty per cent ryania granules were used in 3 replications and 40 % ryania dust in the remaining 4.
[d] Actual yields from untreated plots.
[e] Differences required for significance at the 5% level.

ulated formulation containing 40% ryania was used on three plantations, and the commonly used 40% ryania dust was used on the remaining four.

Two per cent endrin granules gave much better control than was afforded by the ryania treatments. The effects of ryania dust and granules were averaged together since the average difference between them in control obtained was only 3%. The difference in percentage of joints bored between endrin-treated and ryania-treated or untreated plots was larger than that required for significance at the 5% level.

Data presented in table 3 show that yield increases on endrin-treated plots were about twice as large as those measured in ryania-treated plots. The differences between yields of cane and sugar per acre from endrin-treated and untreated plots were greater than the amounts required for significance at the 5% level. The average difference in yield of cane per acre between endrin- and ryania-treated plots was greater than that required for significance at the 5% level.

1958 DDT Test.—Four biweekly applications of 5% DDT granules were compared with comparable treatments of 2% endrin granules and 40% ryania dust in large plots on Waterford Plantation in St. Charles Parish.

Data given in table 4 show that endrin gave far better control of borers than either of the other insecticides used. The difference in percentage of joints bored between ryania-treated and untreated plots was the only one among all possible comparisons which was not as large as the difference required for significance at the 5% level. Treatment with DDT resulted in a significant increase in borer damage.

Average yield increases of slightly more than 11 tons of cane and more than ½ ton of sugar per acre resulted from four biweekly applications of endrin granules. Relatively small increases in yield followed treatments with ryania. Estimated yields were actually smaller in DDT-treated plots than in check plots which received no insecticide treatment.

1958 Small-Plot Test.—Granulated formulations of several insecticides were compared in a small-plot test conducted on Belleview Plantation near Franklin, Louisiana. (Table 5.)

Table 4.—Summary of results from large-plot insecticide experiment conducted on Waterford Plantation in St. Charles Parish, Louisiana, during 1958.[a]

Treatments[b]	Percentage Joints Bored	Yield Increase or Decrease per Acre	
		Tons of Cane	Pounds of Sugar
DDT 5% granules	50	− 2.0	− 482
Endrin 2% granules	7	11.1	1191
Ryania[c] dust and granules	35	3.2	294
Untreated	32	29.9[d]	4689[d]
Tukey's D[e]	4	5.3	730

[a] Sugarcane variety C.P.44-101, first ratoon. Figures are averages of 4 replications.
[b] Four applications of each insecticide were made at biweekly intervals.
[c] First application with 40% ryania dust; last 3 applications with 40% ryania granules.
[d] Actual yields from untreated plots.
[e] Differences required for significance at the 5% level.

Table 5.—Summary of results from small-plot insecticide experiment conducted on Belleview Plantation near Franklin, Louisiana, during 1958.[a]

Treatments[b]	Per Cent Joints Bored	Yield Increase or Decrease per Acre	
		Tons of Cane	Pounds of Sugar
Endrin 2% granules	5	6.1	1353
Thiodan 5% granules	5	5.0	1026
Sevin 5% granules	26	3.9	632
Heptachlor 5% granules	16	3.4	463
Trithion 5% granules	32	3.8	784
DDT 5% granules	47	− 1.0	− 564
Ryania 40% granules	32	2.8	421
Ryania 30% granules	32	2.8	417
Untreated	40	24.5[c]	5649[c]
Tukey's D[d]	10	4.4	1044

[a] Sugarcane variety C.P.44-101, first ratoon crop. Figures are averages of 6 replications.
[b] Four applications of each insecticide were made at biweekly intervals
[c] Actual yields from untreated plots.
[d] Differences required for significance at the 5% level.

Four biweekly applications of endrin, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), heptachlor, and Sevin® (1-naphthyl-*N*-methyl carbamate), respectively, caused appreciable reductions in borer damage. Treatments with Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) or ryania suppressed borer damage to some extent. DDT was apparently responsible for an increase in borer damage. The injury suffered in plots treated with endrin, Thiodan, heptachlor, and Sevin differed significantly at the 5% level from that found in untreated plots. Endrin and Thiodan gave significantly better control than any other insecticide treatments.

Significant increases in yield of cane and/or sugar per acre followed treatment with endrin and Thiodan, respectively. Favorable responses in yield were measured following treatment with all of the other insecticides used except DDT, which was apparently responsible for some decrease in yield.

DISCUSSION.—The weight of accumulated data points conclusively to the fact that endrin is a far better chemical for controlling the sugarcane borer than any other that has been so thoroughly tested at this time. For this reason it is the only insecticide currently recommended by the Louisiana Agricultural Experiment Station for borer control on sugarcane.

Four biweekly applications of 2% endrin granules broadcast at the rate of 12 pounds per acre have given very good control of second and third generation cane borers. During 1958 the average total cost of four airplane applications of endrin granules was slightly less than $10.00 per acre. This means to the grower that he must obtain an increase of slightly more than 1 ton of standard cane per acre to pay for the cost of treatment. The data reported herein show average yield increases ranging from 6 to 11 tons of cane per acre following treatment with endrin.

Though endrin is now widely accepted by Louisiana sugarcane growers as the best control for the sugarcane borer, there was early opposition to its use by some who feared that the use of an organic insecticide for borer

control in Louisiana's Sugar Belt might eliminate some important natural enemies of the borer and thereby increase the overall problem of borer control. Data presented above for DDT and other data collected by Long *et al.* (1958) show that such upsets can and have occurred following insecticide use in sugarcane fields.

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Techniques for Rearing the Chigger Mite, *Trombicula akamushi* (Brumpt)[1]

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ABSTRACT

Methods are described for the laboratory colonization of the chigger mite, *Trombicula akamushi*. The larvae were fed upon chicks and white rats. The eggs of the collembolan, *Sinella curviseta* Brook, served as food for the postlarval stages. A sack for the attachment of the larval mites to the wings of chicks is described and a capsule for the attachment of larvae to rats is described and illustrated. Apparatus for housing chicks while the larval mites are feeding is illustrated and briefly described. A description is also given of methods for the rearing of *S. curviseta*.

Methods for the rearing of the chigger mite, *Trombicula akamushi* (Brumpt), have been described by Sasa & Miura (1953) and Ito *et al.* (1957). Sasa and Miura fed the postlarval stages on *Culex* eggs, while the larval mites were fed on baby rats. Their technique for the attachment of larval mites occasionally permitted the escape of these forms and only a small proportion of larvae usually succeeded in feeding. The same limitations applied to the techniques employed by Ito *et al.* who used white rats as well as mice of the genus *Microtus* as larval hosts, and dragonfly eggs as food for the postlarval stages.

Our colony was initiated with larvae collected from the rat, *Rattus jalorensis* (Bonhote), trapped in the vicinity of Subang, Selangor State, Malaya, in August 1956. Baby chicks and white rats were used as hosts for the larvae, whereas the postlarval stages were maintained on the eggs of the collembolan, *Sinella curviseta* Brook, for four complete generations.

METHODS APPLICABLE TO ALL STAGES.—Two types of containers were used to maintain our cultures. For large numbers of chigger mites (20 or more), we used a 60-ml. wide-mouth, round glass bottle with a black plastic screw-cap. Individual chigger mites were reared in a 1-dram wide-mouth glass vial with plastic insert cap. Both types of containers were prepared by placing a three-quarter inch layer of a plaster-charcoal mixture, modified after Wharton (1946), in the bottom. The mixture which we found most satisfactory was prepared from 225 gms. of artificial dental stone, 5 gms. of Norit-A (decolorizing carbon)[2] and 95 ml. of water. After the wet plaster-charcoal mixture was poured, air bubbles were removed by tapping the container lightly, and the mixture was then permitted to harden. To prepare containers which had dried out, the plaster-charcoal mixture was moistened with tap water, a drop at a time until absorption began to take place very slowly. All containers were kept tightly stoppered to prevent the escape of mites. These containers received sufficient ventilation when food was added. The postlarval stages were fed eggs of the collembolan, *S. curviseta*, daily at the approximate rate of 10 eggs per nymph and 20 eggs per adult. All cultures were maintained at a constant temperature of 27° C. Humidity was not constant because the substrate in the containers was moistened once or twice each week. Transfer of chigger mites from one container to another when required, was accomplished by using a very fine wetted camel's-hair brush. A single container was usually satisfactory for rearing *T. akamushi* through a complete generation.

Mold frequently developed in both types of containers resulting in an increase in the mortality rate in eggs, unengorged larvae and quiescent nymphs. We scraped the mold from the substrate as often as required.

METHOD FOR ATTACHMENT OF LARVAE TO ANIMALS.—For simple maintenance of uninfected chigger mite colonies, attachments were made by placing larvae directly on the plucked back of a 2- to 3-day-old chick. The larvae were first transferred from the culture vial to a syracuse watch glass containing a detergent solution consisting of one drop of commercial liquid detergent in 100 ml. of water, and to the back of the chick with Pasteur pipette.

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[2] Manufactured by Fisher Scientific Company, Fair Lawn, N. J.

This solution was also used for the recovery of larvae from host animals as described by Lipovsky (1951b).

To minimize the loss of larvae, we fastened a silk bolting cloth sack over the wing of a baby chick. The opening was hemmed and provided with draw strings. A sack 2 inches long and 1⅜ inches wide at its mouth fitted the wing of a 2- or 3-day-old white leghorn chick. The primary wing feathers were plucked and some of the down feathers removed with animal clippers before the sack was placed on the wing. Larvae were transferred to the wing by a pipette and the sack was sealed to the wing with Davol Surgical Appliance Cement.[3] The chick was held in a container for 40 hours. The sack was then cut and the larvae removed.

The container which we used for holding host chicks is illustrated in figure 1. It was constructed from a 10-inch length of 7-inch lucite tubing. The lid was made from a circular sheet of lucite, perforated for ventilation, and fitted with screws. Troughs were attached to the cylinder to provide for water and feed. A circular sheet of ¼-inch hardware cloth resting on six lucite supports formed the floor of the container. The container was placed on a stainless steel rack as shown in figure 1. An 8-inch disc of window screening was placed between the rack and container. The assembled apparatus was then placed in an enameled tray containing approximately an inch of the detergent solution.

When larvae were attached to rodents, a capsule was used to confine the chiggers to the host. This capsule was prepared by forcing a ⅜-inch length of ½-inch glass tubing into a 10-ml. ungraduated celluloid centrifuge tube, leaving ⅛ inch of the glass tubing protruding from the mouth of the centrifuge tube. The bottom of the celluloid tube was trimmed flush with the end of the glass section. Six holes were burned in the lip of the centrifuge tube with a hot dissecting needle. A ¼-inch wide rubber ring cut from ¼-inch foam rubber sheeting was slipped over the celluloid tube close to the lip (fig. 2a). Using a curved suture needle and silk suture the rubber ring was sewn to the capsule, figures 2a, 2b.

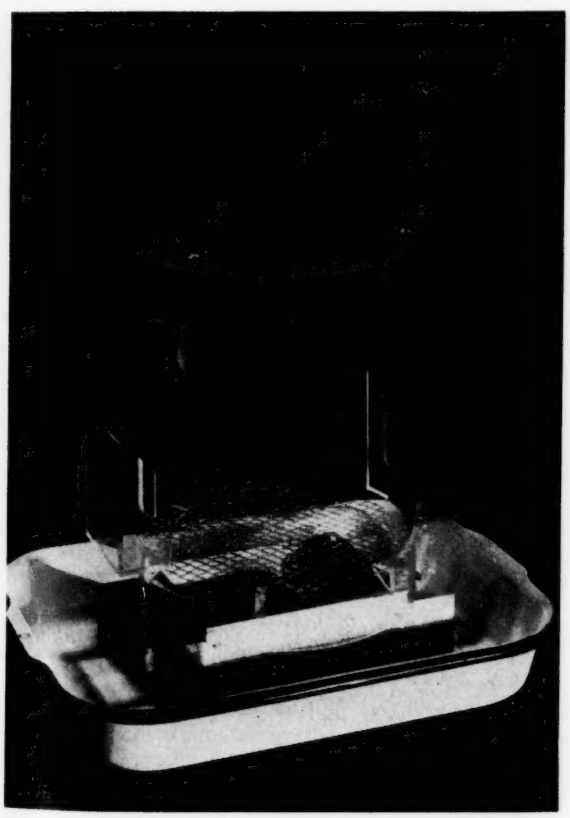

FIG. 1.—Chick cage and equipment for chigger mite recovery.

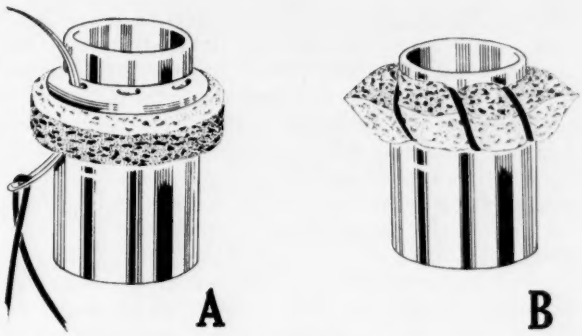

FIG. 2. (a & b)—Capsule for confining chigger mites on rats, showing method of preparation.

To attach the capsule to a white rat, the animal was first anaesthetized with sodium pentobarbital and the hair removed with animal clippers from the dorsal scapular and lateral shoulder areas. The capsule was placed between the two shoulder blades and its position was marked with ink. Six to eight purse string loops,[4] evenly spaced around the periphery of the inked circle were sewn into the skin of the rat, using silk suture and a curved suture needle. The capsule was replaced on the inked circle and a suture wire was inserted through the loops. The suture wire was then fastened around the capsule just above the rubber ring. The slack was next taken out of the loops and the loose suture ends tied together securely. Excess wire and thread were cut away and surgical appliance cement was applied to the junction of the rubber ring and the rat's skin. Finally, a circular hole was cut in a 2- by 3-inch piece of adhesive plaster and the hole fitted over the capsule so that the plaster covered the entire area surrounding the capsule. A plastic insert type cap from a 1-dram wide-mouth vial served as a stopper for the capsule. The rat with attached capsule was placed in a stainless steel shoebox-type cage fitted with legs 1 inch high. The flooring of the cage was made from ¼-inch hardware cloth to which a subflooring of window screening was fixed. The cage was set in a white enamelled tray partially filled with the detergent solution. A small hole was bored in the stainless steel lid to accommodate the neck of a water bottle.

METHODS FOR REARING *Sinella curviseta*.—Eggs of this collembolan were first used as food for the postlarval stages of chigger mites by Lipovsky (1951a). Our method for the laboratory culture of this collembolan follows: A half-pint round cardboard ice cream container was lined with a 12-inch square of aluminum foil. The container

[3] Distributed by Davol Rubber Company, Providence, R. I.

[4] See Stedman's Medical Dictionary, Williams and Wilkins Company, Baltimore, Md., 19th edit., 1957, p. 1347.

was filled to a depth of 1 inch with plaster-charcoal mixture, as previously described for the chigger mite containers. A flat bottomed bottle, approximately 2½ inches in diameter, was wetted and pressed into the plaster until the plaster extrudes to the rim of the ice cream container. A layer of plaster was left on the bottom of the container. After the plaster had set the bottle was removed and the excess foil cut away. Sufficient water was added to the container to give the plaster a wet appearance and thereafter 4 or 5 drops of water were added daily. Each container was started with approximately 150 Collembola. Approximately 0.2 gm. of dry activated yeast was placed in the center of the container daily to provide food. A small sheet of Saran wrap or cellophane was placed between container and lid to minimize the loss of moisture. A culture of this type usually produced eggs for only 4 days. However, if the Collembola were then transferred to a fresh container, egg laying resumed.

ACKNOWLEDGEMENTS—The authors wish to express their appreciation to Dr. J. R. Audy, Institute for Medical Research and Lt. Colonel R. Traub, United States Army Medical Research Unit, Institute for Medical Research, Kuala Lumpur, Malaya for providing the material used to establish the laboratory strain of *T. akamushi*. We are also indebted to Mr. J. R. Evans, Department of Experimental Surgery, Walter Reed Army Institute of Research, for assistance in developing the technique for fastening capsules on rats.

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Experiments in Apple Insect Control[1]

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ABSTRACT

Eleven combinations of insecticides and fungicides were applied to McIntosh apples in Connecticut for control of insects and diseases in 1957 and 1958. When these were rated on the basis of pest control, quality of fruit, safety, and cost, the order of preference was (1) Guthion-captan, (2) Sevin-Kelthane-captan, (3) methoxychlor-glyodin-ferbam, and (4) lead arsenate-malathion-captan. Kepone-captan, used only in 1958, gave very promising results. The possible place of these and other pesticides in apple insect control is discussed. Insecticides with trademarked names mentioned above are: Guthion® *O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate; Kelthane® 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol; Kepone (1189) formerly known as a chlorinated polycyclic ketone, now designated Decachloro-tetrahydro-4,7-methanoindenone; and Sevin® 1-naphthyl-*N*-methyl carbamate.

Results given in this paper are from experiments conducted in Connecticut during 1957 and 1958. Tables 1 and 2 give results of trials of 11 different combinations designed to give reasonable control of pests compared with standard treatments. Untreated fruit was not available in 1957, because none was left on the trees at harvest time. In addition, tables 3 and 4 give estimates of the effect of sprays on insects obtained in the ground cover in 1958. Surveys were also made in commercial orchards in which Guthion®*O'O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate was used.

METHODS.—Some of the factors used in evaluating sprays were as follows:

Percentage clean reflects both insect and disease control and is a measure of the growers' requirements. It may change from year to year depending on the weather and on the effect of sprays on the abundance of parasites and predators. If one major pest is eliminated, another may increase in abundance.

Quality is important. If the color is off as it may be following some treatments, if the sugar content is low as when mites are not controlled, and especially if the finish is ruined, quality is so poor that good control of pests has done little good. Effect on flavor must also be considered.

Safety is another important consideration. Some growers will not use highly poisonous insecticides.

Costs. No matter how good a spray combination is, there isn't much chance that it will be generally used if it is too expensive. However, this factor may be difficult to determine depending on indirect benefits or harmfulness. For example, lack of control may affect production as well as reduce the value of a crop. Some insecticides or fungicides contain nutrients which tend to increase production.

RESULTS.—In 1957, estimates based on the factors outlined were equal for lead arsenate-malathion-captan and Guthion-thiram, with other combinations less desirable. In 1958 the most promising combinations based on cost,

[1]Accepted for publication January 30, 1959.

Table 1.—Results of treatments against pests of McIntosh apples. 1958.

TREATMENT[a]	DILUTION IN POUNDS OR PINTS PER 100 GAL.	PER CENT CLEAN EXCEPT FOR APPLE MAGGOT	PER CENT WITH APPLE MAGGOT
Untreated	—	0.3	high
Lead arsenate-ryania-captan	3–6–1.5	48.3	10.8
Delnav-captan	2–1.5	65.6	11.1
Lead arsenate-malathion-captan	3–2–1.5	64.8	5.6
Methoxychlor-glyodin-ferbam	3–2 pts.–1.5	82.2	1.3
Kepone-captan	2–1.5	83.2	4.9
Sevin-Kelthane-captan	2–1.5–1.5	84.3	1.2
Guthion-captan	2–1.5	90.3	1.5

[a] Formulations of materials used in tables 1–4. Captan (50%) w. p.; Delnav (25%) w. p.; ethion (25%) w. p.; ferbam (76%) w. p.; Guthion (12.5% or 15%) w. p.; glyodin (34%) liquid; Kelthane (18.5%) w. p.; Kepone (50%) w. p.; lead arsenate (100%) w. p.; methoxychlor (50%) w. p.; ryania (95%) w. p. (table 1), (50%) w. p. (tables 2, 3); Sevin (50%) w. p.

A total of 10 applications were made during the season, the first without insecticide and the last without fungicide. High pressure hydraulic equipment was used in all tests except those reported in table 4.

insect and disease control, fruit finish, and operator safety were; (1) Guthion-captan, (2) Sevin-Kelthane-captan, (3) methoxychlor-glyodin-ferbam, (4) Lead arsenate-malathion-captan, in the order given. Sevin® is 1-naphthyl-*N*-methyl carbamate; Kelthane® is 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol.

The insecticide components of the mixtures seemed to have the following characteristics based on studies of the data accumulated:

Guthion proved to be a useful universal insecticide with few weaknesses and a good record as far as phytotoxicity and fruit finish were concerned. Combinations with several fungicides produced little injury and no russeting of the fruit. Flavor tests were promising but incomplete. Destruction of parasites and predators appeared to be less severe than with chlorinated hydrocarbons (tables 3 and 4). Data given in table 3 are based on a representative collection made at a time when parasites and predators were most numerous.

Sevin produced some surprising results such as control of the apple aphid (*Aphis pomi* DeG.) and the rosy apple aphid (*Anuraphis roseus* Baker) and general pest control including that of the apple maggot *Rhagoletis pomonella* (Walsh). Its weaknesses are few and they may be easily remedied. Combined with Kelthane and captan, Sevin produced clean fruit of reasonably good quality. As in the case of Guthion, flavor tests are not yet complete. Its safety and high tolerance appeal to many. Cost of the Sevin-Kelthane captan spray appears to be a little high.

Kepone® (1189) (decachloro-tetrahydro-4,7-methanoindenone), known for at least 7 years, was studied carefully in 1958. In combination with captan, it gave little or no injury to foliage, but its main value seemed to be in the lack of destruction of beneficial Hymenoptera, and other insects (table 3). Considering its efficiency, it seemed to be much better in this respect than ryania. No flavor tests have been completed to date.

Table 2.—Results of treatments against pests of McIntosh apples. 1957.

TREATMENT[a]	POUNDS PER 100 GALS.	PER CENT CLEAN EXCEPT FOR APPLE MAGGOT	PER CENT WITH APPLE MAGGOT
Untreated[b]			
Lead arsenate-ryania-captan	3–1.5–1.5	48.0	14.2
Ryania-captan	6–1.5	54.6	13.3
Ethion-thiram	2–1.5	57.2	11.6
Ethion-captan	2–2	60.4	9.0
Lead arsenate-malathion-captan	3–2–1.5	65.6	6.3
Guthion-captan	2–1.5	83.1	.3
Guthion-thiram	2–1.5	91.2	.5

[a] For formulations used see footnote a of table 1. Total of 11 applications.
[b] No records obtained because there was no fruit.

Table 3.—Ratios of harmful to beneficial insects from ground sweeps and percentage of Hymenoptera parasitica[a] on Aug. 15, 1958.

TREATMENT[b]	RATIO	HYMENOPTERA PARASITICA (%)
Ryania-lead arsenate-captan	3.3	15.4
Kepone-captan	4.7	15.7
Lead arsenate-malathion-captan	5.5	12.4
Delnav-captan	7.2	8.9
Guthion-captan	9.3	7.8
Methoxychlor-glyodin-ferbam	11.8	6.9

[a] Hymenoptera parasitica represents forms identified as parasitic in habits, such as aphid parasites. By harmful is meant phytophagous species plus scavengers. The ratio is obtained by dividing the predator-parasites into the harmful group thus: $\frac{\text{Phytophagous, etc.}}{\text{Predator-parasites}}$. Treatments were replicated four times. A total of 120 sweeps for each treatment were made; 30 in each plot.
[b] A total of 10 applications were made in 1958 at 10-day to 2-week intervals, the first without insecticide and the last without fungicide.

Ryania combinations, after several years, appeared to be about the same as at the beginning; namely, poor general insect control (low percentage of clean fruit) in spite of its lack of destruction of beneficial forms. Combined with lead arsenate, it lowered the efficiency of lead arsenate in our tests. Apparently its rather mild insecticidal action is not enough for our area. Flavor tests have given generally good results. It is probably the safest insecticide that can be used, from the operator's standpoint.

Lead arsenate continued to be reasonably effective except against the European apple sawfly (*Hoplocampa testudinea* (Klug)), the mites (*Tetranychus telarius* (L.) and *Panonychus ulmi* (Koch)), the apple aphid, the rosy apple aphid, and the white apple leafhopper (*Typhlocyba pomaria* McA.). Combinations with malathion to remedy these defects have not been completely successful because mites have developed resistance as in many other localities. In spite of this, lead arsenate is still widely used in

Table 4.—Ratios of harmful to beneficial insects in ground sweeps from commercial orchards treated with Guthion or with chlorinated hydrocarbon insecticides.[a]

ORCHARD NO.	GUTHION[b]	CHLORINATED HYDROCARBONS (TDE-DDT-DIELDRIN)
1	9.0	59.0
2	2.6	19.4
3	5.5	5.8
4	9.6	17.0

[a] Based on an average of two collections made in late summer after most of the spray program was completed.
[b] Several different fungicides were used with Guthion in the different orchards. Regular commercial spray programs were followed mostly and applications were made with hydraulic or air-blast equipment at normal dilutions. Applications in orchard No. 3 were made with a mist blower at much higher concentrations and the spray was applied with little drip onto the ground cover.

Connecticut, partly because of its low cost and good control of the apple maggot. Color and finish of the fruit is affected somewhat adversely. The material has some incompatibilities, such as that with glyodin, which necessitate caution. Its action on beneficial insects has been very mild, almost the same as ryania, but mites and San Jose scale (*Aspidiotus perniciosus* Comst.) remained troublesome even when malathion was added. The flavor has been good when used on the McIntosh variety but the adhesiveness of this material make residues a problem.

Methoxychlor was used mainly for control of the plum curculio (*Conotrachelus nenuphar* (Hbst.)) and has given good results. Combinations with glyodin and ferbam were not satisfactory in 1958 because of the rough finish which resulted from their use. Other combinations have been better. The methoxychlor-malathion-captan combination used in tests not reported herein can evidently be improved by adding calcium carbonate, although leaf-spotting on both apples and peaches was not prevented entirely. In spite of its long period of usefulness, there seems to be some question regarding the effect of methoxychlor on the flavor of the fruit.

Ethion (formerly called Nialate or Niagara 1240) and *Delnav*®(2,3-p-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate). Action of these two insecticides seemed more specific than that of the others discussed herein so that the overall control was not generally adequate. They may prove useful, however, for control of specific pests.

Pienus Infestation on True Firs in Western Washington[1]

NORMAN E. JOHNSON[2]

ABSTRACT

This is a first report of an undescribed chermid of the genus *Pineus* attacking both the bole and branches of *Abies amabilis* and *A. grandis* with little apparent damage. It also distinguishes this insect from an apparently similar balsam woolly aphid (*Chermes piceae* Ratz.) that severely damages these same true firs. *Pineus* first instar larvae when settled on bark secrete long, irregular wax threads but retain activity up to molting, and in crowns does not cause galling of branch tips. *Chermes* with short, regular wax ribbons becomes inactive, and causes "gouting" of branch tips.

A chermid of the genus *Pineus* has been found attacking Pacific silver and grand fir (*Abies amabilis* and *A. grandis*) in Lewis County, Washington. To the unaided eye this insect appears very similar to the balsam woolly aphid (*Chermes piceae* Ratz.) which is causing serious damage to mature true fir trees on several hundred thousand acres in western Washington and Oregon (Johnson & Wright 1957, Rudinsky 1957). The occurrence of *Pineus* in areas as yet unaffected by the balsam woolly aphid could confuse the accurate reporting of the range and spread of both of these insects. The purpose of this paper is to report the occurrence of *Pineus* on true fir in the Pacific Northwest and to describe characteristics that will distinguish it from the balsam woolly aphid.

Published reports indicate that *Pineus* usually attacks only pines or spruces. However, Essig (1926) reports that *Chermes pinicorticis* Fitch (synonymous with *Pineus strobi*) "apparently is the common species on spruce, pine and fir from British Columbia to California." However, it is not known what tree species are included in the general term "fir". Annand (1928) does not report species of *Pineus* as attacking trees other than pine or spruce. Keen (1952) lists two species of *Pineus*, occurring in the forests of the Pacific Northwest whose alternate hosts are not known.

Specimens of this chermid were sent to the U. S. National Museum for identification. Miss Louise Russell, assigned the specimens to the genus *Pineus*; however, she advised that specific identification must await collection of forms occurring on the primary host. She reported that to her knowledge this is the first record of a member of this genus attacking true fir. This was confirmed by

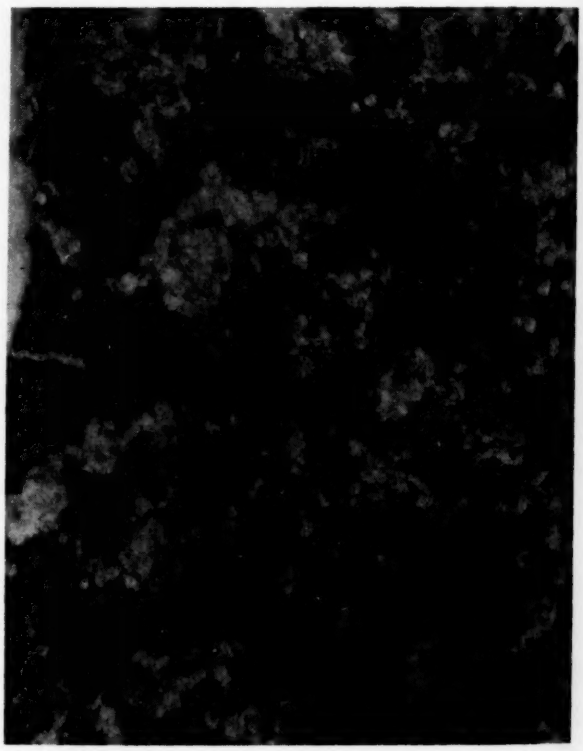

FIG. 1.—Wax wool of *Pineus* on the bark of grand fir.

[1] Accepted for publication December 9, 1958.

[2] Entomologist, Weyerhaeuser Timber Company Forestry Research Center Centralia, Washington.

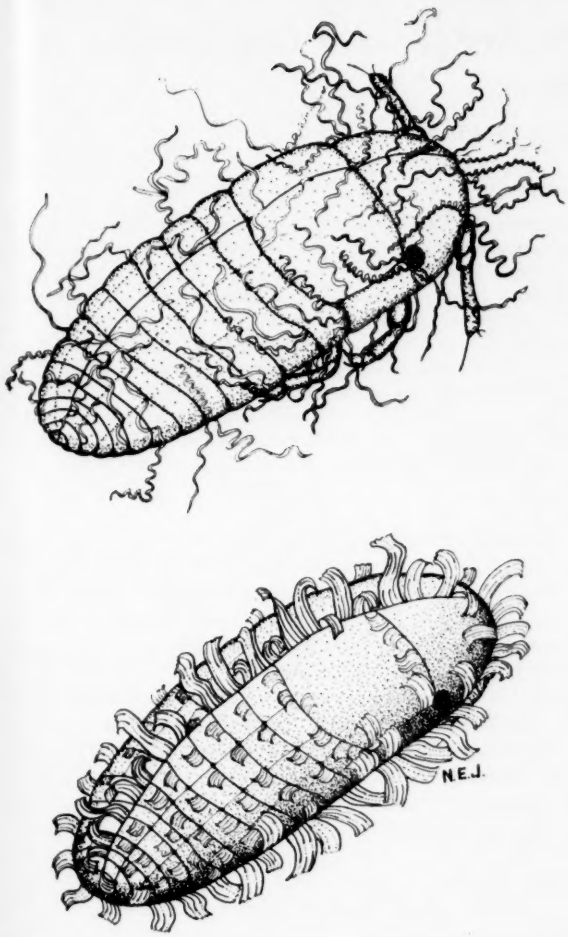

FIG. 2.—Above: *Pineus* with long thin, irregular wax threads Below: The balsam woolly aphid with short, flat, regularly arranged wax ribbons

other authorities.[3] Field observations indicated that *Pineus* is common on Pacific silver fir on McDonald Tree Farm in the vicinity of Curtis, Washington and on grand fir in Borst Park, Centralia, Washington. This chermid attacks both the bole and branches of the host. Bole infestations have the cottony appearance similar to that of the balsam woolly aphid (fig. 1). Field examinations indicate that *Pineus* does not cause galling of the branch tips or what Balch (1952) terms "gouting" in the case of the balsam woolly aphid. This is substantiated by laboratory studies in which two-year-old seedlings of Pacific silver fir and noble fir (*Abies procera*) were infested with this *Pineus* and the balsam woolly aphid. Those seedlings successfully infested with the balsam woolly aphid produced conspicuous swellings while those infested with *Pineus* showed no galling.

Distinctive characteristics separating these two species of chermids under low magnification is the wax secretion of the first instar larvae that have settled on the bark (fig. 2). The first instar of *Pineus* produces long, thin wax threads, whereas the balsam woolly aphid produces wax ribbons that are conspicuous around the margin of the body and along the mesal line. The balsam woolly aphid after inserting its stylets folds its antennae and legs beneath its body and becomes closely appressed to the bark. By contrast, *Pineus* evidences more activity by movement of antennae and legs even up to the time of molting. The adult *Pineus* is more circular in shape than the balsam woolly aphid, and the eggs and larvae are lighter in color. Specimens of the two genera when cleared and placed on slides can be distinguished by the number of abdominal spiracles (Annand 1928, Varty 1956).

Pineus does not appear to be harmful to its host tree in western Washington, whereas currently, the balsam woolly aphid is considered the most serious insect enemy of true firs in the Pacific Northwest. Because of the similarity in appearance of these closely related insects, it is important to distinguish them in the field in order to avoid confusion in the reporting and subsequent treating of infestations.

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[3] Correspondence with Miss Russell of the U. S. Department of Agriculture, July 28, 1958; Dr. O. Eichhorn of the University of Freiburg, August 13, 1958; and Dr. I. W. Varty, Canada Forest Biology Division, Fredricton, N. B., September 10, 1958.

Distribution and Host Range of Eight Species of *Trogoderma* in California[1]

R. G. Strong,[2] G. T. Okumura,[3] and D. E. Sbur[2]

Abstract

Maps are used to indicate the known distribution of eight species of *Trogoderma* (*T. glabrum* (Herbst), *T. granarium* Everts, *T. grassmani* Beal, *T. inclusum* LeConte, *T. ornatum* (Say), *T. parabile* Beal, *T. simplex* Jayne, and *T. sternale* Jayne) in California based on infestations recorded in the California Khapra Beetle Survey. The authors carefully note, however, that most infestations of *T. granarium* have been eradicated and the known infestations, at the time of acceptance of their manuscript, would probably be fumigated by the time the paper was published.

Of 132 host materials listed from survey reports, *Trogoderma glabrum* was found in association with 6, *T. granarium* with 37, *T. grassmani* with 13, *T. inclusum* with 33, *T. ornatum* with 20, *T. parabile* with 77, *T. simplex* with 60, and *T. sternale* with 79. Results from preliminary food tests in the laboratory with *T. granarium* and *T. parabile* are given, and the known food habits of *T. granarium*, the species which has received the most attention, is presented as a summary of several published reports.

The need for detailed investigations on *Trogoderma* is apparent from the distribution and broad host associations recorded for the eight species in California and by the general lack of information on *Trogoderma* as storage pests in the United States.

Emergence of *Trogoderma* from comparative obscurity in the United States was marked by the identification (Allen & Linsley 1954) of the khapra beetle, *Trogoderma granarium* Everts, from stored wheat and barley in Tulare County, California, in October of 1953. Owing to the importance of the khapra beetle as a storage pest in other parts of the world, its positive identification in California led to an immediate search for infestations in this State and a keen awareness of *Trogoderma* spp. in other sections of the country.

Establishment of the Khapra Beetle Interior Quarantine in January 1955 by the Bureau of Plant Quarantine of the California Department of Agriculture, with plans for the eradication of the pest, intensified inspections for khapra. The difficulty of separating species and the necessity for absolute identification required that all *Trogoderma* specimens collected in the California Khapra Beetle Survey be examined by systematic entomologists[4] for positive identification. Gammon (1958) gives a complete account of activities and division of responsibility in the khapra beetle eradication program in which the U. S. Department of Agriculture, Khapra Beetle Control Project, the California Department of Agriculture, Bureau of Entomology, and the County Commissioners of Agriculture jointly share responsibility for the khapra beetle detection survey in California.

Keifer (1958) has summarized identification totals for specimens collected during the survey for khapra beetles in 1957, and these are representative of the enormous task performed by systematic entomologists in the khapra beetle eradication program.

As an aid in the identification of khapra larvae and adults, Okumura & Blanc (1955) prepared an illustrated key to species of *Trogoderma* and related genera of Dermestidae based on the work of Hinton (1945), Beal (1954), and Howe & Burges (1955). Later, Beal (1956) revised his keys to neartic species of *Trogoderma* and enlarged his account of the food habits of the species found in the United States. Thus, over the 5-year period of the survey summarized as a part of this report, records on the occurrence of *Trogoderma* in California are based on reliable identification of specimens.

Unlike most other dermestids, the khapra beetle prefers grain and cereal products to substances of animal origin but will develop on various host materials (Hinton 1945) Because of this and other known habits of the beetle, survey inspections have been concerned primarily with sites more or less intimately connected with commerce (grain storage and milling facilities, feed yards, staple foods and seed warehouses, and ranches—to mention a few) owing to the possibility of more rapid dissemination from such infestations. Yet, natural sources, habitats, and reservoirs of storage pests so aptly described in Linsley's comprehensive reports (1942, 1944) are being considered.

Strong & Okumura (1958) reviewed records in the files of the Bureau of Entomology, California Department of Agriculture, to prepare a list of insects and mites associated with stored foods and seeds in California. The general distribution and broad host range indicated in their report for eight species of *Trogoderma* prompted comparisons between species with respect to distribution and hosts in order to evaluate their relative importance as storage pests and, in turn, determine the need for investigations on the biology and control of several species similar to the work reported on the khapra beetle by Lindgren *et al.* (1955), and Lindgren & Vincent (1959).

The purpose of this paper is to give the known distribution of eight species of *Trogoderma* in California, the hosts with which these were associated at the time of collection (if any), preliminary results from food experiments with certain species in the laboratory, plus a limited review of references in the literature on the food habits of *Trogoderma*. The information is given in a manner suitable for rapid comparisons between species.

Distribution.—Based on collection and identification records from the California Khapra Beetle Survey, the known distribution of eight species of *Trogoderma* in California has been summarized as presented in figures 1 and

[1] Paper No. 1131, University of California Citrus Experiment Station. This paper is a joint contribution from the University of California, Citrus Experiment Station, Department of Entomology, and the California Department of Agriculture, Division of Plant Industry. Accepted for publication March 2 1959.

[2] University of California, Riverside.

[3] California Department of Agriculture, Sacramento.

[4] Inquiries concerning the status of the khapra beetle eradication program in California should be addressed to R. W. Harper, Chief, Bureau of Entomology California Department of Agriculture, Sacramento.

FIG. 1.—Known distribution of *Trogoderma* in California: (1) *Trogoderma glabrum* (Herbst), (2) *Trogoderma granarium* Everts (See also footnote 4), (3) *Trogoderma grassmani* Beal, (4) *Trogoderma inclusum* LeConte.

2. It should be noted that infestations of *Trogoderma* found at sites inspected in the survey for khapra beetles were probably most often, but not always, the result of unnatural distribution.

One infestation was sufficient to indicate the presence of a species in a county, but the relative abundance of a species in any one county is not shown. However, the relative abundance within the State is indicated from a summary of the distribution of *Trogoderma* species by counties, the figures indicating the numbers of counties

FIG. 2.—Known distribution of *Trogoderma* in California: (5) *Trogoderma ornatum* (Say), (6) *Trogoderma parabile* Beal, (7) *Trogoderma simplex* Jayne, (8) *Trogoderma sternale* Jayne.

with recorded infestations: *glabrum*, 10; *granarium*, 18; *grassmani*, 12; *inclusum*, 33; *ornatum*, 27; *parabile*, 41; *simplex*, 40; *sternale*, 45.

The distribution and host range of *T. granarium* (the khapra beetle) has been included in this paper primarily to provide a basis for comparisons between species. At the time of acceptance for publication, five known infestations remained in the State, four in Riverside County, and one in Madera County, and by the time of publication these probably will have been fumigated. No attempt

was made to determine whether infestations would persist at any particular site, or the population density that might be reached by a specific infestation. Nor was the frequency of collection of a species summarized for this paper, although such information would suggest the true range of species within California.

As indicated by distribution maps (fig. 1 (1 to 3)), *Trogoderma glabrum*, *T. granarium*, and *T. grassmani* apparently prefer the environments of inland valleys and desert areas while *T. inclusum* (fig. 1 (4)), *T. parabile* (fig. 2 (6)), *T. simplex* (fig. 2 (7)), and *T. sternale* (fig. 2 (8)) are found generally throughout the State. *Trogoderma ornatum* was collected most often from the coastal areas, but as seen in figure 2 (5), infestations were found in many outlying counties.

In regard to the distribution of *Trogoderma* in California, climatic conditions are of basic importance and extremes are found in this State, even within some counties. However, discussion of the distribution of storage insects on a climatic basis must be deemphasized where storage environments are independent of temperature, particularly when buildings are artificially heated or insects cause the heating of commodities.

HOST RANGE.—*Survey Results.*—The nature of the khapra beetle survey leaves little chance for *Trogoderma* to escape detection when inspections are made. All possible hiding places and host materials are examined intensely. Host associations are recorded when specimens are collected for identification, and the hosts or host materials with which *Trogoderma* spp. have been found in California are given in table 1.

Food habits of eight species of *Trogoderma* are indicated by the host index presented in table 1, but it should be noted that occurrence of *Trogoderma* with the hosts listed merely indicates that specimens may be found on the various materials, all of which are not necessarily primary hosts for the species concerned. In this report, primary hosts are considered to be those foods on which *Trogoderma* populations increase as the result of larval feeding, development to adults, and reproduction. Secondary hosts are considered to be foods on which larvae persist, but usually do not reach adult maturity. Thus, the necessity for using the term "host association."

The range in host associations of the eight species is somewhat similar to the distribution of species by counties. Of the 132 host materials listed in table 1, *Trogoderma glabrum* was found in association with 6, *T. granarium* with 37, *T. grassmani* with 13, *T. inclusum* with 33, *T. ornatum* with 20, *T. parabile* with 77, *T. simplex* with 60, and *T. sternale* with 79.

It is suggested from further analysis of host associations that *Trogoderma granarium*, *T. glabrum*, and *T. grassmani* prefer cereal grains and seeds, or their products, whereas the other species are found with a more general host association. The occurrence of several species on flower and vegetable seeds is of particular interest since these seeds are produced in limited quantities and insect injury could result in serious economic losses. Association of *T. parabile*, *T. simplex*, *T. sternale*, and other species of *Trogoderma* to a lesser degree, with dried fruits and nuts and grocery commodities is probably of more economic importance than has been recognized to the present time.

Laboratory Food Studies With Trogoderma granarium and parabile.—As suggested by the range of host associations in table 1, many problems are involved in the development and use of control and preventive measures for *Trogoderma*. However, the usefulness of a knowledge of its primary hosts is beyond question in considering such measures. Locating sources of infestations is a fundamental step in any storage sanitation program, and these sources are most often dependent on foods upon which insects develop and reproduce.

Many records are available on the occurrence of *Trogoderma* feeding on various materials, but for other than *T. granarium*, little information exists on the primary host range of the *Trogoderma* found in California. For this reason, preliminary food studies with *T. granarium* and *T. parabile* were made to permit direct comparisons between two species in order to gain an insight into the need for more detailed studies with all species of *Trogoderma* found in the state.

First instar larvae of *Trogoderma granarium* and *T. parabile* were placed on 29 host materials (each species handled separately) in cloth-covered glass vials and held at 90° F. To each vial containing 5 grams of food material, 50 larvae were added. Four vials, giving a total of 200 larvae for each species, were used in the evaluation of each material. Test vials were examined at weekly intervals and pupae were removed as they appeared. When adults emerged, they were placed on the same kind of foods upon which the larvae fed and held for observations on reproduction. From the results of these tests presented in table 2, similarities and differences in the food habits of *T. granarium* and *T. parabile* are obvious, within the limits prescribed by the foods used.

DISCUSSION.—*Distribution.*—No doubt the known distribution of *Trogoderma* in California (figures 1 and 2) will be extended by further inspections, especially in the northern counties of California. However, one species, *T. granarium*, will probably be eliminated by a successful eradication program. As mentioned earlier, at the time the manuscript was accepted only two counties, Madera and Riverside, had known infestations of the khapra beetle and these will probably be fumigated before publication of this paper. But, according to Howe & Lindgren (1957), new infestations are likely to appear for several years, for this insect is not easily killed by cold or starvation, and it is difficult to find when present in small numbers. The latter point makes the khapra beetle an awkward inspection problem for port quarantine inspectors, especially those of California and Texas, and emphasizes the chance of the pests being reintroduced after completion of a successful eradication campaign, unless more efficient methods of detection and identification become available.

No attempt was made to determine persistence of infestations or population densities in the California survey for khapra. However, on occasion infestations of *Trogoderma grassmani*, *T. inclusum*, *T. parabile*, *T. simplex*, and *T. sternale* have been observed in association with grain storage, and in feed processing and storage facilities with population densities reminiscent of *T. granarium*. *Trogoderma glabrum* was seldom found in large numbers at any location and infestations apparently do not persist. *Trogoderma ornatum* infestations tend to persist, but population levels are seldom high.

Table 1.—Host or possible host materials with which *Trogoderma* spp. were associated at the time of collection during the California Khapra Beetle Survey.[a]

Host Material	*Trogoderma* spp.							
	glabrum	*granarium*	*grassmani*	*inclusum*	*ornatum*	*parabile*	*simplex*	*sternale*
Cereal Grains and Seeds								
Alfalfa		×	×	×		×	×	×
Austrian peas						×		
Barley	×	×	×	×	×	×	×	×
Beans		×	×	×	×	×	×	×
Beet (field and sugar)		×	×		×	×	×	×
Bentgrass								×
Bermuda grass								×
Bluegrass								×
Brome						×		×
Broomcorn				×	×			
Burnet						×		
Cabbage								×
Cactus					×			
Canary grass		×						
Carrot						×		
Cauliflower								×
Clover		×		×		×		×
Coriander								×
Corn (field)	×	×			×	×	×	×
Corn (pop)							×	
Cotton		×	×			×	×	×
Cucumber						×		×
Dallis grass						×		
Dandelion						×		
Eggplant						×		×
Fenugreek				×				
Fescue						×	×	×
Flax		×	×				×	×
Garbanzos					×	×		
Kohlrabi								×
Lettuce						×	×	×
Millet		×		×	×	×	×	×
Milo		×		×		×	×	×
Muskmelon						×	×	×
Mustard								×
Nasturtium								×
Oats		×		×		×	×	
Onion			×			×	×	
Pea (garden)		×		×		×		×
Pepper						×	×	×
Poppy (Oriental)		×						
Pumpkin						×		×
Rice		×		×		×	×	×
Rye						×		
Ryegrass						×	×	×
Safflower				×		×	×	×
Sesame							×	
Sesbania							×	×
Snapdragon								×
Sorghum		×	×				×	×
Soybeans						×		
Spinach			×			×		×
Squash						×	×	
Stocks								×
Sudan		×	×	×		×	×	×
Sunflower						×	×	×
Sweet corn						×		
Swiss chard		×						
Tomato		×				×	×	
Trefoil								×
Vetch		×		×		×	×	×
Watermelon		×		×		×	×	
Wheat	×	×	×	×	×	×	×	×
Wheatgrass						×		×
Animal Feeds (Plant Origin Only)								
Alfalfa hay		×						×
Alfalfa meal				×				
Cottonseed hulls							×	×
Cottonseed meal		×		×	×		×	×
Linseed meal								×
Rolled barley		×			×		×	×
Rolled milo		×						
Rolled oats							×	×
Rice bran						×		
Rice meal							×	
Rice screenings								×
Soybean meal	×			×			×	
Sugar beet pulp		×		×				×
Wheat bran	×			×			×	×
Mixed Animal Feeds, Mashes and Pellets								
Calf						×		
Dog						×	×	×
Goat							×	
Cattle (dairy)		×		×	×	×	×	×
Hog						×		
Horse							×	
Poultry		×	×		×	×	×	×
Rabbit					×	×	×	×
Dried Fruit and Nuts								
Almond meats						×	×	×
Figs								×

Table 1.—(*continued*)

Host Material	*Trogoderma* spp.							
	glabrum	*granarium*	*grassmani*	*inclutum*	*ornatum*	*parabile*	*simplex*	*sternale*
Peaches						×		
Peanuts							×	×
Pecan meats							×	×
Prunes				×				×
Raisins						×	×	×
Walnut meats		×			×	×	×	×
Grocery Commodities								
Beef bullion cubes						×		
Candy (chocolate and nuts)			×			×		×
Cereals (breakfast foods)				×		×	×	×
Chicken soup (dehydrated)						×		
Cookies						×		
Corn meal		×		×		×	×	×
Crackers (soda)				×				×
Eggs (dry powdered)							×	
Egg noodles						×		
Flour and mixes						×		×
Fudge mix						×		
Garlic (dry bulb)				×				
Hominy grits						×		
Macaroni		×				×		×
Milk (dry powdered)						×	×	×
Pudding mixes						×		
Rice		×		×		×	×	×
Spaghetti						×		
Spices						×		
Sugar		×		×				
Peppers, dry pod						×		×
Wild rice				×	×	×		
Miscellaneous								
Almond hulls (bulk and crushed)							×	×
Bird seed						×	×	
Blood meal							×	
Chicken manure		×						
Date pollen						×	×	
Fish meal						×	×	
Meat scraps (dry)								×
Oat straw		×						
Tortillas (cooked, dry)						×		
Wollen clothing								×
Natural Hosts (or Habitats)								
Ant nest		×						
Beehives				×	×		×	×
Mud dauber nests	×			×	×	×	×	×
Spider egg masses						×		×
Spider webs					×	×	×	×
Wasp nests				×	×			×

[a] Summary of hosts recorded over a 5-year period. X indicates species was collected from host material one or more times.

Food Habits.—Detailed studies on the food habits of *Trogoderma* are limited, but Hopkins (1955), Lindgren *et al.* (1955), and Lindgren & Vincent (1959) have reported results from tests with *T. granarium* and results are presented in this paper (table 2) from preliminary tests with *T. granarium* and *T. parabile.* Noon (1958) also reported on the food habits of the khapra beetle and gave results from experiments in which materials were included that might serve as hosts in natural reservoirs for *T. granarium.* Unfortunately, in the latter report, the author failed to mention reasons for the high mortality of larvae in most of his tests, although the data presented suggested that extraneous influences were involved, perhaps disease-producing protozoa similar to those found by Marzke & Dicke (1958) in several species of *Trogoderma.*

The known food habits of *Trogoderma granarium* are summarized in table 3 by combining information from the first three references cited in the preceding paragraph with results from food tests presented in this paper. The

Table 2.—Development of *Trogoderma granarium* and *T. parabile* on various foods at 90° F. during minimum observational periods of 145 days.

Food	*granarium*		*parabile*	
	Egg to Adult (Days)	Status at End of Observations	Egg to Adult (Days)	Status at End of Observations
Wheat recleaned whole grain	43	Reproduction	40	Reproduction
Cracked wheat	33	Reproduction	40	Reproduction
Wheat germ	31	Reproduction	36	Reproduction
Whole wheat flour	29	Reproduction	41	Reproduction
Barley recleaned whole grain	38	Reproduction	60	Reproduction
Rolled barley	41	Reproduction	35	Reproduction
Barley cereal, pre-cooked for babies	157	No reproduction	75	Reproduction
Shelled corn, recleaned whole kernel	42	Reproduction	40	Reproduction
Ground corn	36	Reproduction	36	Reproduction
Yellow corn meal	32	Reproduction	61	Reproduction
Corn cereal, pre-cooked for babies	—	Few larvae alive	—	Larvae
Whole brown rice	41	Reproduction	43	Reproduction
Cracked brown rice	33	Reproduction	44	Reproduction
Whole white rice	37	Reproduction	—	Larvae
Cracked white rice	42	Reproduction	—	Larvae
Instant white rice	—	Few small larvae alive	—	Very small larvae
Oatmeal	43	Reproduction	49	Reproduction
Oatmeal, instant	43	Reproduction	46	Reproduction
Oatmeal, pre-cooked for babies	65	Reproduction	55	Reproduction
Pablum, pre-cooked mixed cereals	48	Reproduction	56	Reproduction
Pinto beans	120	Reproduction	—	Larvae in cracked beans
Lima beans	145	No reproduction	—	Small larvae
Flax seed	—	Few larvae alive	—	Larvae
Ground dog food	37	Reproduction	44	Reproduction
Seedless raisins	—	Larvae died	—	Larvae died
Powdered skim milk	—	Small larvae	—	Larvae
Brewer's yeast	81	No reproduction	—	Larvae
Copra meal	—	Few larvae alive	—	Larvae
Gelatin	—	Larvae died	—	Larvae died

summary is given with subheadings similar to those used in table 1 to facilitate comparisons between host associations found in the survey for khapra beetles and the known food habits of *T. granarium* based on results of laboratory tests, and for general comparisons with other species. Where any doubt exists on the status of a host material it is listed as a secondary rather than as a primary host. Each host material has been listed under the heading considered most appropriate, and only once, although some materials might easily be placed in at least two categories—for example, garbanzos could be listed under both cereal grains and seed and grocery commodities. Since *Trogoderma* adults live only a short time and are not known to feed, all reference to food habits is concerned with larvae. By comparing the known food habits of *T. granarium* as presented in table 3 with the host associations listed in table 1, the areas where further efforts are needed on food studies with this species are seen readily.

Seven species of *Trogoderma* (*T. glabrum*, *T. granarium*, *T. grassmani*, *T. inclusum*, *T. parabile*, *T. simplex*, and *T. sternale*) have been reared successfully on ground barley, ground corn, ground wheat, ground dog food, and laying mash in cultures of the University of California Department of Entomology Stored-Products Insects Laboratory at Riverside. This indicates that cereal grains and mixed animal feeds are primary hosts of these species, although only *T. granarium* and *T. parabile* have been included in laboratory studies to date.

COMMENTS.—In considering the distribution and host range of eight species of *Trogoderma* in California, one is impressed by the lack of information on *Trogoderma* as storage pests in the United States, and the need for biology and control investigations on the eight species found in California is apparent from their recorded distribution and host associations. Furthermore, in the event of reintroduction of the khapra beetle after eradication, sufficient information on other species of *Trogoderma* should be available to aid inspectors in locating infestations of khapra more efficiently and taxonomists in making more rapid determination of specimens.

Table 3.—Summary of the known food habits of *Trogoderma granarium* based on results from several reports[a] on food tests in the laboratory.

PRIMARY HOSTS	SECONDARY HOSTS
Cereal Grains and Seeds	
Barley	Alfalfa seed
Black-eyed beans	Castor beans
Corn	Cottonseed, fuzzy[b]
Garbanzos	Flax seed[b]
Lentils	Lima beans[b]
Oats	Pinto beans[b]
Peas	
Rice	
Sorghum	
Soybeans	
Wheat	
Animal Feeds and Concentrates	
Barley, rolled and ground	Alfalfa hay
Corn, ground	Beef blood, dried
Dog food, ground	Copra meal
Oats, rolled	Cottonseed meal
Orange pulp, dried	
Rice, ground	
Wheat-bran, cracked, ground	
Dried Fruits and Nuts	
Almond meats	Dates
Peanuts	Figs
Pecan meats	Peaches
Walnut meats	
	Pears
	Prunes
	Raisins
Grocery Commodities	
Bread	Barley cereal, pre-cooked for babies
Coconuts, dry	Chocolate, chips and powdered
Corn meal	Corn cereal, pre-cooked for babies
Crackers	Egg noodles[b]
Flour, white and whole wheat	Instant rice
Hominy grits	Nutmeg
Oatmeal, regular, instant, and pre-cooked for babies	Skim milk, powdered[b]
Pablum, pre-cooked mixed cereals	Spaghetti[b]
Pearl barley	Sugar
	Tapioca
Wheat germ	
Miscellaneous	
	Brewer's yeast
	Wool yarn

[a] Differentiating hosts as primary and secondary is entirely the responsibility of the authors of this paper and was based on conclusions drawn by them after a careful review of results from tests reported by Hopkins (1955), Lindgren *et al.* (1955), Lindgren & Vincent (1959), and those found in table 2 of this paper.

[b] Certain reported observations indicate that these materials may sometimes be considered as primary hosts.

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Control of Insects Injurious to Birdsfoot Trefoil in New York[1]

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ABSTRACT

A series of three control experiments was conducted to evaluate the effectiveness of various insecticides for the control of insects injurious to birdsfoot trefoil, *Lotus corniculatus* L., grown for hay and seed.

Of the materials tested for control of the lepidopterous leafroller, *Sparaganothis xanthoides* Walker, parathion at 0.5 lb. per acre was found to be highly effective and the most economical to use.

The application of lindane at 0.25 lb. per acre for the control of the meadow spittlebug, *Philaenus leucophthalmus* (L.), resulted in significant increases in yields of forage and seed. Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) applied at the rate of 1.0 lb. per acre as a pre-bloom treatment also gave significant increases in yields of forage and seed. Parathion and aldrin applied at 1.0 lb. per acre as a pre-bloom treatment both gave significant increases in the number of legumes produced.

Birdsfoot trefoil, *Lotus corniculatus* L., has become an important perennial forage legume in much of the Northeast, especially in areas inherently of low fertility and poor drainage. The increasing use of birdsfoot trefoil as a forage and seed crop and the recognition that a large number of injurious insects are associated with it in New York have led to the need for control measures applicable to that crop.

The first extensive work in the United States concerned with insects injurious to birdsfoot trefoil was that of Neunzig & Gyrisco (1955), who reviewed earlier scattered reports of insects feeding on birdsfoot trefoil and gave an account of their research on the number of insects found to be injurious to birdsfoot trefoil in New York. Marshall (1951) noted that trefoil was one of the preferred hosts of the meadow spittlebug, *Philaenus leucophthalmus* (L.). Neunzig *et al.* (1958) reported results of studies with the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze), and the tarnished plant bug, *Lygus lineolaris* (P. deB.), in which they demonstrated that plant bugs could be responsible for blasting of flower buds and "bud drop" on birdsfoot trefoil, resulting in decreased seed yields. Ridgway & Gyrisco (1957) reported that various insecticidal treatments, on the average, resulted in significant increases in yields of seed from birdsfoot trefoil. MacCollom (1958), reporting on 2 years of study, found that early-season applications of insecticides with good residual properties resulted in significant increases in yields of seed. He suggested that the principal insects involved were the meadow spittlebug and several species of the family Miridae.

This paper is a progress report of three field experiments that were designed to control the several species of

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insects thought to be the most important in decreasing seed and forage yields of birdsfoot trefoil.

METHODS AND MATERIALS.—Malathion, parathion, Phosdrin,® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) endrin, aldrin, heptachlor, methoxychlor, lindane and Thiodan®(6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) were applied alone or in various combinations as emulsions to 20×50 foot plots that were randomized within blocks and replicated from three to six times (see tables 1 through 4). Each plot was surrounded by a 5-foot border strip and each block was separated by a 30-foot border strip to prevent contamination by drift. All the insecticides were applied with a truck-mounted, low pressure, low gallonage "Yellow Devil" sprayer at 40 p.s.i. of pressure at the rate of 20 gallons of spray per acre. The commercial sprayer had an 18-foot brush-type boom mounted with 11 No. 8002 *T-jet* nozzles.

Experiment 1.—The lepidopterous leafroller, *Sparganothis xanthoides* Walker, can cause extensive feeding damage to birdsfoot trefoil and can prevent pollination and legume setting by webbing of the terminals and blooms. A series of insecticides that were tested for the control of this caterpillar were applied on June 20, 1958, in a field of birdsfoot trefoil near Georgetown, New York. The insecticides used, rates at which they were applied in pounds per acre, and means of the number of larvae counted in each of 2 square feet in each plot appear in table 1. Counts of caterpillars were made on June 23, 1958, by arbitrarily selecting 2 square feet in each plot where trefoil was prevalent. The foliage was removed from each square foot and examined for the presence of leafrollers (see table 1). The differences between means were tested for significance using Tukey's honestly significant difference test. Of all the insecticides tested, parathion and Phosdrin were outstanding. Although Phosdrin was as effective as parathion at the same dosage level it is not economically competitive with the older established insecticide. The half-pound dosage appeared to be adequate for effective leafroller control.

Experiment 2.—The insects most likely to be responsible for decreased seed yields of birdsfoot trefoil are the meadow spittlebug and the various plant bugs. This experiment was directed primarily toward the control of these pests. The insecticides that were used were lindane and Thiodan at 0.25 lb. and 1 lb. per acre of actual toxicant, respectively. Lindane was selected for the control of the meadow spittlebug because it is a proven material for that purpose (Marshall 1951). Although Thiodan is not generally known as a particularly good material for plant bug control, its use in 1957 showed promise for increasing yields of birdsfoot trefoil (Ridgway & Gyrisco 1957), and it was used for that reason. The treatments (see table 2) and the untreated check formed a factorial arrangement. They were applied in fields of the varieties Empire and Viking. The lindane was applied on May 17, 1958, for control of the meadow spittlebug; the Thiodan was applied on June 16, 1958, for control of plant bugs and other members of the insect complex.

Table 1.—Results of tests with various insecticides to control *Sparganothis xanthoides*. Georgetown, N. Y. 1958.

TREATMENT	POUNDS OF ACTUAL TOXICANT PER ACRE	NO. OF LARVAE	
		Transformed[a] Means	Untransformed Means
Phosdrin	0.5	0.71[b]	0.0
Parathion	1.0	0.71[b]	0.0
Parathion	0.5	0.88[b]	0.27
Methoxychlor	2.0	1.62[b]	2.12
Heptachlor	1.0	1.86[b]	2.96
Endrin	0.25	2.40	5.26
Heptachlor	0.5	3.10	9.11
Malathion	1.0	3.32	10.25
Untreated	—	4.00	15.50

[a] The insect counts were transformed prior to statistical analysis by using the transformation $\sqrt{X+0.5}$ where X is the number of insects per plot.

[b] Significantly different from the untreated at the 5% level using Tukey's honestly significant difference test. Tukey's D is 2.13.

Table 2.—Control of tarnished plant bug with lindane and Thiodan on birdsfoot trefoil Aurora, N. Y. 1958.

TREATMENT	POUNDS OF ACTUAL TOXICANT PER ACRE	NO. OF TARNISHED PLANT BUGS	
		Transformed Means[a]	Untransformed Means
Thiodan+lindane	1.0 0.25	3.26	10.13
Thiodan	1.0	2.41	5.13
Lindane	0.25	4.42	19.04
Untreated	—	4.82	22.75

[a] Thiodan treatments resulted in decreases in the number of tarnished plant bugs according to the F test at the 5% level. The transformation as described in footnote "a" of table 1 was also used here.

Spittlebug counts, which were taken on June 16, 1958, by counting the number of spittle masses present in a given unit area in each plot, indicated that the lindane application gave essentially complete control of the meadow spittlebug.

Counts of tarnished plant bugs were taken on June 17, 1958, by recording the number of insects taken in 50 sweeps of a 15-inch diameter insect net from each plot. These data are presented in table 2.

Yields of forage were taken on July 2, 1958, by cutting and weighing the foliage from a swath 39 inches wide and 15 feet long in each plot. Yields of birdsfoot trefoil seed were taken on July 31, 1958, from a mowed swath 39 inches wide and 30 feet long through the middle of each plot. The foliage was air dried and put through a custom-built, beating-type thresher, after which the seed was cleaned and weighed. The mean weights of the forage and seed yields from each treatment are included in table 3.

The results of the statistical analysis indicate that forage yields were increased significantly by treatment with lindane in early spring and by treatment with Thiodan in mid-June. The results also indicate that the same treatments gave significant increases in seed yields.

Precise conclusions as to which insects of the insect complex were controlled by the applications of insecticides are difficult to make, but insect counts made during the course of the experiment showed that the insects present in the largest numbers in the untreated check were the meadow spittlebug and the tarnished plant bug. Lindane gave nearly complete control of the meadow spittlebug and Thiodan gave effective control of the tarnished plant bug. Since these insects occurred in the largest num-

Table 3.—Forage and seed yields of birdsfoot trefoil to which lindane and Thiodan were applied.[a] Aurora, N. Y. 1958.

Treatment	Pounds of Actual Toxicant per Acre	Mean Yields of	
		Forage[b]	Seed[c]
Thiodan+lindane	1.0 0.25	22.8	144
Thiodan	1.0	21.3	91
Lindane	0.25	21.7	74
Untreated	—	18.7	58

[a] The forage and seed yields were significantly increased according to the F test at the 5% level.
[b] Pounds (green weight) from a 39′×15′ strip per plot.
[c] Grams from a 39′×30′ strip per plot.

bers and were controlled, they were believed to have been responsible for most of the damage.

Experiment 3.—This experiment was primarily concerned with the effects of pre-bloom insecticidal treatments upon the yield of birdsfoot trefoil seed. The insecticides and rates that were used were selected because they are suggested frequently for the control of plant bugs (see table 4). The treatments were applied on June 17, 1958, to a crop of birdsfoot trefoil of the variety Empire, which had just begun to bloom and which had been treated in early spring with toxaphene for control of the meadow spittlebug.

Table 4.—Increases in number of legumes produced from insecticide applications to birdsfoot trefoil grown for seed production. Albany County, New York. 1958.

Treatment	Pounds of Actual toxicant per Acre	Mean No. of Legumes in 6 Sq. Ft./Plot
Parathion	1.0	665.0[a]
Aldrin	1.0	662.7[a]
Endrin	0.25	549.2
Malathion	1.0	520.8
Untreated	—	392.5

[a] Significantly different from the untreated at the 5% level, using Tukey's h. s. d. test. Tukey's D is 169.0.

Yields of birdsfoot trefoil seed were estimated on August 2, 1958, by counting the number of mature legumes in each of 6 square feet that were selected in a systematic manner in each plot. The means of these counts appear in table 4 with the respective treatments. The data were analyzed, and the differences between means were tested for significance using Tukey's honestly significant difference test.

Applications of parathion and aldrin resulted in significant increases in the number of legumes produced. Treatments with malathion or endrin resulted in considerable increases in seed yield but variability in stand between some of these plots was too great to prove significance. However, it is believed that all of the insecticides tested are practical in controlling plant bugs in fields of birdsfoot trefoil grown for seed. The data presented here and other data collected in 1958 suggest that the use of numbers of legumes to determine seed yields offers a speedy and practical method of determining the effectiveness of insecticides for increasing seed yields.

CONCLUSIONS.—From the work conducted to date, the data suggest to the birdsfoot trefoil seed producer an early spring application for control of the meadow spittlebug and a pre-bloom treatment to control plant bugs and other insects of the insect complex.

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Experiments on the Control of Fall Armyworm[1] in Sweet Corn

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ABSTRACT

In 1957 an experiment was conducted using granulated DDT for control of fall armyworm, *Laphygma frugiperda* (J. E. Smith). Treatments consisted of one to five weekly applications which did not give satisfactory control.

In 1958 two experiments were conducted, one with granulated DDT and one with DDT emulsion sprays. More satisfactory control was obtained with granulated DDT in 1958 than in 1957, but control was not reflected in yield or number of marketable ears as opposed to nubbins or ear size. In the experiment using DDT emulsion sprays, DDT remained constant in all treatments, and the volume of water was varied. One quart of DDT emulsifiable concentrate in 100 gallons of water was more effective than 1 quart in 50 or 25 gallons of water.

One of the most discouraging factors in the production of late sweet corn in Maryland is the usual infestation of fall armyworm, *Laphygma frugiperda* (J. E. Smith). Larvae attack corn from the time the plants appear above ground until the ears are ready for harvest. Adequate insecticidal coverage, particularly of young rapidly growing plants, is difficult to maintain. Brett (1953) and Blanchard *et al.* (1955) have demonstrated the effectiveness of DDT in controlling this insect. Because of a need for control methods that are economical and effective,

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Table 1.—Treatment schedules, infestation of fall armyworm larvae during the period of weekly treatments, number of larvae, larval tunnels and yields of corn at harvest, treated with 20 pounds of granulated DDT (7.5%), 1957.

	Treatments					Infestation (Larvae/100 Plants)				Larvae/100 Stalks at Harvest	Stalks Tunneled (Angle)[a]	Yield (Lb. per Plot)
	8/15	8/22	8/29	9/5	9/12	8/28	9/4	9/11	9/18			
1.	No treatment					29.2	56.6	48.3	38.4	15.9	34.80	14.9
2.	×					20.0	44.2	38.3	32.6	15.0	30.63	17.3
3.	×	×				25.8	25.0	25.8	20.2	12.5	31.98	18.8
4.	×	×	×			—	38.4	32.4	17.5	7.5	28.91	15.8
5.	×	×	×	×		—	—	25.8	18.2	4.2	20.58	14.7
6.	×	×	×	×	×	—	—	—	16.3	2.5	16.72	15.9
	L.S.D. @ 1% level										11.12	n.s.

[a] Percentages transformed to angles for statistical analyses.

several experiments were conducted in 1957 and 1958 at the Maryland Experiment Station for the control of this insect on sweet corn with DDT sprays and granulated formulations.

In 1957 a randomized block experiment was carried out for preliminary evaluation of granulated DDT and to obtain information on the number of treatments required. Victory Golden sweet corn was planted July 15 in four-row plots 40 feet long. On August 15, when the plants were 6 to 8 inches high and infested with many small larvae, the first treatments were applied. Treatments consisted of one to five weekly applications (see table 1) of 20 pounds of granulated DDT (7.5%) on 30–60 mesh Attaclay with a Gandy Hi-Or-Lo spreader-ette. From the beginning of treatment until harvest, fall armyworm populations remained at a fairly high level. Table 1 summarizes the results of this experiment. One row from each plot was harvested. It is apparent that treatment reduced infestation, but even in the plots receiving five treatments, the infestation level was only 57% lower than in the untreated check 1 week after the last treatment. At the time of harvest on October 3 the stalks from the harvested rows were cut, stripped, and examined for live larvae and larval tunnels. A significant reduction occurred in number of tunnels but yields were not significantly increased by treatments.

Two experiments were conducted in 1958, one with granulated DDT and one with DDT emulsion sprays. The experiment with granulated DDT was designed to compare number of applications, effectiveness of early versus last pretasseling applications, and to learn the effect of the treatments on yields under the 1958 population levels. Deep Gold sweet corn was planted July 1, 1958. Plots in this experiment were 80 feet long and four rows wide. Infestation of plants by small larvae was first noticed about July 15. On July 26 when approximately 50% of the plants were infested, the first treatments were applied. The treatment schedules are shown in table 2 with a summary of the results of these treatments. Unfortunately, weather conditions delayed an infestation count after the last applications until August 22, when there were too few larvae to reflect results of treatment. When silking began, all plots, including checks, were sprayed twice for corn earworm control. One row from each plot was harvested on September 18. At harvest the marketable ears were counted separately from the nubbins, or small ears, to determine if the pretassel injury influenced yield, ear size, or number of unmarketable ears. Analysis of covariance indicated no effect of treatment on total yield or number and weight of marketable ears at the 1958 population levels. Neither was there an increase in the size of marketable ears. After the ears had been harvested a 25-plant sample was taken from each plot and examined for larvae and tunnels. Living larvae and tunnels were found in insignificant numbers in the stalks even from check plots.

In the second experiment, DDT emulsion sprays were applied at the constant rate of 1 quart of 25% emulsifiable concentrate per acre in varying amounts of water to determine if increasing the spray volume would increase control because of more complete whorl penetration. The corn was planted on July 22; pretasseling applications of all treatments were made on August 2 and August 15 when the plants were 2 and 3 feet high. After treatments

Table 2.—Corn yields from plots treated for fall armyworm and average number of fall armyworm larvae per 100 plants in plots of sweet corn treated with 20 pounds of granulated DDT (7.5%), 1958.

	Treatments Applied on			No. Larvae/100 Plants on		Pounds per Plot	Per Cent Good Ears by		Weight per Ear (Lb.)
	7/26	8/1	8/8	7/31	8/7		Weight	Number	
1.	No treatment			29.3	30.8	43.3	79.0	72.4	0.49
2.	×			8.0	25.3	37.3	89.1	80.4	.48
3.	×	×		21.0	2.8	38.2	77.6	69.1	.46
4.	×	×	×	10.6	2.8	40.2	81.1	68.9	.45
5.		×		66.8	5.3	34.1	70.1	52.4	.44
6.			×	52.0	20.0	46.6	83.8	70.7	.46
7.		×	×	48.0	9.3	36.6	79.3	66.9	.44
						n.s.	n.s.	n.s.	n.s.

Table 3.—Fall armyworm infestation on sweet corn treated with 1 quart of 25% DDT emulsifiable concentrate in varying amounts of water.[a]

Water (gallons)	Infestation on Aug. 20		Infestation on Sept. 2	
	Larvae/100 Plants	Per Cent Reduction	Larvae/100 Plants	Per Cent Reduction
25	4.0	86.9	69.0	30.7
50	5.5	81.9	62.0	37.7
100	2.0	93.4	59.0	40.7
Untreated	30.5	—	99.5	—

[a] Treatments applied August 2 and 15, 1958.

were completed counts were made to determine the degree of control. The treatments and infestations are shown in table 3. One quart of DDT was slightly more effective against larvae in the whorl when applied in 100 gallons of water per acre than in 50 or 25 gallons.

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Bioassays of Weathered Residues of Several Organophosphorus Insecticides[1]

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Abstract

Spray residues of Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), malathion, and methyl parathion on cotton plants were weathered by several natural or simulated climatological conditions and bioassayed with the boll weevil, *Anthonomus grandis* Boh. All the experiments were conducted in the laboratory.

Exposure to temperatures above 100° F. for 4 to 6 hours during a 24-hour period had little or no effect on the residual toxicities of Guthion, malathion, or methyl parathion to the boll weevil. However, toxic residues of malathion and methyl parathion were affected adversely by exposure to high natural light intensities of the magnitude of 13,000 foot-candles at elevated temperatures. Residues of Guthion were affected adversely by simulated rain, but were not affected by high temperatures.

Some data are presented indicating that organophosphorus and chlorinated hydrocarbon insecticides can be combined to increase the toxicity of the residues to the boll weevil.

The effects of temperature and humidity variations, simulated wind and rain on toxic residues of Guthion,® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), malathion and methyl parathion on cotton plants as determined by bioassay methods were reported by Hightower & Martin (1958).

The object of the present study was to determine the effects of elevated temperatures and high natural light intensities on the residual toxicities of the above-mentioned materials to the boll weevil, *Anthonomus grandis* Boh. Toxaphene and Sevin®(1-naphthyl-*N*-methyl carbamate) were included in some of the tests for comparison. Two experiments were conducted also to explore the possibility of using mixtures of organophosphorus and chlorinated hydrocarbon compounds to extend the period of insecticidal activity of the residues.

Materials and Methods.—All the experiments were conducted in the laboratory. Natural or simulated climatic conditions were used for weathering the residues, depending upon the nature of the experiment. The treated plants exposed to high temperatures were held in an uninsulated room with a metal roof for 24 hours. Temperatures rose to 100° to 110° F. for 4 to 6 hours during the 24-hour delay periods. The light intensities in the room varied from 500 to 800 foot-candles. Plants exposed to high natural light intensities were held outside the laboratory for 24 hours in an open-top cellulose acetate cage.

Boll weevils were reared in the laboratory from field-collected cotton squares. The temperature in the rearing room was held constant at 85° F. The adult weevils were fed at least 3 days on white cotton blooms prior to each bioassay test.

Commercial oil emulsion concentrates of all the insecticides, except Sevin, were diluted with water and applied as sprays to potted cotton plants. Sevin was applied as a suspension spray. The plants were passed through the spray at a speed of 5 m.p.h. by a turntable device. Two hollow-cone nozzles operating under a pressure of 60 p.s.i. delivered a volume of liquid equivalent to a field dosage of 5 gallons of spray per acre. Dosages were expressed in terms of pounds of toxicant per acre.

In order to measure the weathering effects of the selected climatic factors acting on residues for a certain period of time, plants were treated and divided into the following categories:

Immediate Release: Plants on which insects were released 30 to 45 minutes after treatment.

24-Hour Delay Control: Treated plants which were held for 24 hours in the laboratory at an average temperature of 85° F., and an average relative humidity of 60%

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Table 1.—Effects of simulated rain and high natural light intensities at high temperatures on the residual toxicities of Guthion, Sevin, and toxaphene to the boll weevil.[a]

Treatment	Pounds Active Ingredient/A.	Average Per Cent Control[b]				Per Cent Reduction in Toxicity		
			24-Hour Delay			24-Hour Delay		
							Additional Loss of Residual Toxicity Owing to	
		Immediate Release	Control	High Temperature; High Light Intensity	0.5″ Rain	Control	High Temperature; High Light Intensity	0.5″ Rain
Toxaphene	3.0	52	50	29	26	0	42	48
Guthion	0.125	100	91	92	49	9	0	46
Sevin	1.0	84	76	77	77	9	0	0

[a] Environmental conditions during 24-hour exposure period:
24-hour control and control plus 0.5 inch simulated rain.
Temperature: Maximum 86° F., Minimum 70° F.
Relative humidity: Maximum 92%, Minimum 66%.
24-hour delay including high natural light intensities at high temperatures.
Temperature: Maximum 100° F., Minimum 76° F.
Relative humidity: Maximum 96%, Minimum 46%.
Maximum light intensities: 11,000–13,000 foot candles.

[b] Unbroken lines denote no significance by Duncan's multiple range test.

prior to the release of the weevils on them. Light intensities in the holding room varied between 500 and 800 foot-candles.

24-Hour Delay Plus Weathering: Plants which were exposed to the specified weathering conditions for all or part of the 24-hour prerelease period.

Four plants were used for each treatment and the treatments were replicated four times in each experiment. Ten boll weevils were released on each plant. After the weevils were released on the plants, the caged plants were kept in the holding room at 85° F. An average percentage control based on the third-day mortality count was calculated by Abbott's formula. An analysis of variance was made on each test and individual insecticide means under each weathering condition were compared by a multiple range test (Duncan, 1955). These comparisons are shown in the tables as horizonal lines under the average percentage control figures. Unbroken lines denote no statistical significance between means at the 5% level.

RESULTS.—*Experiment 1:* Although simulated rain drastically reduced the residual toxicity of Guthion, high natural light intensities with coincident elevated temperatures during the exposure period had no appreciable effects on the residues (table 1). Residues of Sevin, applied at a dosage equivalent to 1 pound of toxicant per acre, remained equally toxic to the boll weevil under all the above described weathering conditions. The results obtained with toxaphene varied considerably among the replications.

Experiment 2: High temperatures in the absence of bright sunlight did not reduce the residual toxicities of methyl parathion or malathion to the boll weevil (table 2). Excellent kills were obtained in all instances after exposure of the residues to temperatures ranging from 100 to 110° F. for 4 to 6 hours. The differences among the toxaphene treatments were not significant.

Experiment 3: Toxic residues of malathion and methyl parathion were affected adversely by a 24-hour exposure outside the laboratory during the month of August (table 3). Maximum light intensities of about 13,000 foot-can-

Table 2.—Effect of high temperature on the residual toxicities of methyl parathion, malathion, and toxaphene to the boll weevil.[a]

Treatment	Pounds Active Ingredient per Acre	Average Per Cent Control			Per Cent Reduction in Toxicity	
			24-Hour Delay		24-Hour Delay	
		Immediate Release	Control	High Temperature	Control	Additional Loss of Residual Toxicity Owing to High Temperature
Toxaphene	2.0	28	13	17	54	39
Methyl parathion	0.2	98	88	83	10	15
Malathion	0.5	89	88	87	1	1

[a] Environmental conditions during 24-hour exposure period:
24-hour control
Temperature: Maximum 87° F., Minimum 82° F.
Relative Humidity: Maximum 85%, Minimum 50%.
24-hour high temperature
Temperature: Maximum 110° F., Minimum 80° F.
Relatiive humdity: Maximum 72%, Minimum 30%.

Table 3.—Effect of high natural light intensities on the residual toxicities of malathion, methyl parathion, and toxaphene to the boll weevil.[a]

Treatment	Pounds Active Ingredient per Acre	Average Per Cent Control[b]				Per Cent Reduction in Toxicity		
			24-Hour Delay			24-Hour Delay		
							Additional Loss of Residual Toxicity Owing to	
		Immediate Release	Control	High Temperature Control	High Natural Light Intensities	Control	High Temperature Control	High Natural Light Intensities
Toxaphene	2.0	38	29	25	9	24	14	69
Malathion	0.5	98	98	90	45	0	8	54
Methyl parathion	0.2	99	89	86	34	10	3	61

[a] Environmental conditions during 24-hour exposure period:
24-hour control
Temperature: Maximum 86° F., Minimum 82° F.
Relative humidity: Maximum 74%, Minimum 30%.
24-hour high temperature control
Temperature: Max 112° F., Min. 78° F.
Relative humidity: Maximum 79%, Minimum 32%.
24-hour high temperature and high natural light intensities
Temperature: Maximum 104° F., Minimum 76° F.
Relative humidity: Maximum 98%, Minimum 40%.
Light intensities: Maximum 13,000 foot candles.

[b] Unbroken lines denote no significance by Duncan's multiple range test.

Table 4.—Effect of a 24-hour exposure to outdoor temperature, humidity and light conditions on the residual toxicities of malathion and a mixture of malathion and toxaphene to the boll weevil.

Spray Treatment	Pounds Active Ingredient per Acre	Average Per Cent Control[a]		Per Cent Reduction in Toxicity Owing to
		Immediate Release	24-Hour Delay	24-Hour Delay
Experiment 4[b]				
Malathion	0.5	91	82	10
Malathion +toxaphene	0.5 2.0	89	88	1
Toxaphene	2.0	25	19	24
Experiment 4[c]				
Malathion	0.25	87	9	90
Malathion +toxaphene	0.25 2.0	88	77	13
Toxaphene	2.0	27	15	44

[a] Unbroken lines denote no significance by Duncan's multiple range test.
[b] Environmental conditions during 24-hour exposure period:
Temperature: Maximum 91° F., Minimum 66° F.
Relative humidity: Maximum 98%, Minimum 52%.
Light intensities: 7,000 to 9,500 foot candles.
[c] Environmental conditions during 24-hour exposure period:
Temperature: Maximum 95° F., Minimum 68° F.
Relative humidity: Maximum 98%, Minimum 35%.
Light intensities: 7,000 to 11,000 foot candles.

dles were recorded during these exposures. Maximum temperatures ranged from 90 to 104° F. Residues of these materials on treated plants under similar temperature and humidity conditions in the absence of bright sunlight were as toxic to weevils as the initial deposits of the insecticides. In spite of the relatively low kills obtained with toxaphene under all conditions, a reduction in residual toxicity by high light intensities at elevated temperatures was evident.

Experiment 4: Experiments 4[b] and 4[c] were designed to determine the effect of a 24-hour exposure to outdoor temperature, humidity and light conditions on the residues of a mixture of an organophosphorus compound, and toxaphene. Malathion was arbitrarily selected as a representative of the phosphate insecticides.

When malathion was applied at a dosage equivalent to 0.5 pound of toxicant per acre, the addition of toxaphene did not influence the outcome of the test (table 4). However, when the dosage of malathion was reduced to the equivalent of 0.25 pound of toxicant per acre, the kills obtained on the residues of the mixture were considerably greater than those obtained on the malathion residues alone (table 4). Whether the higher light intensities encountered during the latter experiment influenced these results is a matter of speculation.

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The Spotted Lady Beetle, *Coleomegilla maculata* (De Geer), as a Predator of European Corn Borer Eggs[1]

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ABSTRACT

The spotted lady beetle, *Coleomegilla maculata* (De G.), is a known predator of the eggs of the European corn borer, *Pyrausta nubilalis* (Hbn.), but the actual extent of control had never been determined. The fecula of the spotted lady beetle and other predators found on corn plants were characterized and a method devised for collecting these pellets beneath the site of borer eggs. By examining the fecula, it could be determined whether this beetle had fed. In two different fields under observation 16.5 and 15.3% of the eggs were fed upon.

Studies on food preference and potential egg consumption showed that the spotted lady beetle would feed only upon pollen, pea aphids, *Macrosiphum pisi* (Harr.), or eggs of the European corn borer, when these foods were on appropriate plants, in this case older corn leaves and alfalfa. It was found that the number of eggs an individual adult spotted lady beetle could consume averaged 59.4 per day. Temperature and rainfall had no significant effect upon feeding.

The spotted lady beetle, *Coleomegilla maculata* (De G.), is one of the commonest and most uniformly distributed insects in Delaware. The form which occurs here falls into Timberlake's race *lengi*. It can be found readily on a great variety of plants, is present early in the spring, and maintains a fairly high population late into the fall. Although its food preference is known to be variable, the extent of its host range has not been completely determined. Its recorded food consists of eggs of the European corn borer, *Pyrausta nubilalis* (Hbn.) (Baker *et al.* 1949); the pea aphid, *Macrosiphum pisi* (Harr.); melon aphid, *Aphis gossypii* Glov.; cabbage aphid, *Brevicoryne brassicae* (L.); currant aphid, *Capitophorus ribis* (L.); greenbug, *Toxoptera graminum* (Rond.); German grain aphid, probably *Macrosiphum granarium* (Kby.); chinch bug, *Blissus leucopterus* (Say); eggs of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); and of the bollworm, *Heliothis zea* (Boddie); larvae of the asparagus beetle, *Crioceris asparagi* (L.); and of the cottonwood leaf beetle, *Chrysomela scripta* F.; and corn pollen (Britton 1914). It has been reported by Cody (1927) to feed on the persimmon psylla, *Trioza diospyri* (Ashm.); by Richardson (1915) on a syrphid fly *Mesogramma polita* Say; by Everly (1938) on a mirid *Trigonotilus rubicornis* (Geoffrey); and by Caffrey & Worthley (1927) on both a pentatomid *Podisus placidus* Uhl. and a reduviid *Sinea diadema* Fab. Folsom (1909) reported that it fed on coccinellid eggs and pupae, including eggs of its own kind, as well as on adults of *Coccinella sanguinea* Say. The latter observation seems to have been based on a misidentification. He apparently meant *Cycloneda munda* (Say).

The relative abundance of the spotted lady beetle, its frequent presence on corn plants, and the knowledge that it fed on European corn borer eggs, suggested that it might be an important factor in the biological control of the borer. With this in mind, a study was made of its habits, particularly of those in which it is associated with the European corn borer.

Some investigators of the European corn borer have praised the spotted lady beetle highly as a destroyer of borer eggs, while others did not think it of great value. It was evident that either little attention had been paid to the spotted lady beetle as a predator or that its feeding habits fluctuated considerably from one area to another. Baker *et al.* (1949) credit it with destroying 50% of the eggs at Toledo, Ohio, while others have not been so specific. Crawford & Spencer (1922) indicate that it " . . . probably does more good than any other . . . " and " . . . was repeatedly seen to eat every egg mass it found." Drake (1926) claimed that it occasionally fed on egg clusters. Huber *et al.* (1928) refer to it as devouring eggs, but felt that the percentage destroyed was probably very small. Marston & Dibble (1930) and Caffrey & Worthley (1927) attribute frequent egg feeding to it.

Several predators, including the spotted lady beetle, feed on the eggs of the European corn borer. Among these are the thirteen-spotted lady beetle, *Hippodamia tridecimpunctata* (L.); the convergent lady beetle, *Hippodamia convergens* Guer.; a small red mite *Trombidium fuliginosum* Koch (Bartholomai 1954); *Orius insidiosus* Say and *Nabis ferus* (Linn.) (Beard 1943); and the larvae of an elaterid *Crypotohypnus abbreviatus* Say (Hawkins & Devitt 1953). Bartholomai (1954) also mentions that several species of thrips, chrysopids, and spiders feed upon eggs and larvae to a lesser extent.

Early in the course of these studies it became evident that the larvae of the spotted lady beetle feed on corn borer eggs only to a minor extent. One reason is that this coccinellid apparently lays eggs only at sites of aphid colonies. This apparent fact is supported by the observation that larvae never were found on corn until the corn leaf aphid, *Rhopalosiphum maidis* (Ashm.), appeared. Another reason for the small amount of egg predation by the larvae is their limited mobility. They commence moving about in search of other sources of food only when the aphid colonies become decimated. The larvae were observed feeding on corn borer eggs, but only after the corn leaf aphid had nearly disappeared. For this reason these studies were directed toward the activities of the adult.

METHODS.—*General Observations.*—During the course

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of insect-pest-survey work, observations were made on the activities of the spotted lady beetle over the 2-year period 1957 and 1958. Notes on this beetle were made principally in fields of corn, alfalfa, and clovers. An average of 15 fields of corn were visited weekly between June 1 and September 30 each year.

Food Preference of Caged Adults—Field-collected spotted lady beetle adults were confined in cages and offered, as food, a choice of pea aphids, corn pollen, and European corn borer eggs. The pea aphids were collected in the field as needed. Corn pollen was collected from plants grown in the greenhouse. The borer eggs were obtained from an oviposition chamber constructed according to the description of Briand (1929). Aphids, eggs, and loose pollen were first placed in a small battery jar and a pair of spotted lady beetle adults introduced therein. Later, pollen caked on a fragment of corn leaf was offered to the beetles. Several different arrangments of cages containing a small corn stalk with egg masses pinned to its leaves and a sprig of alfalfa containing pea aphids were tried. In one experiment, a cage was constructed from two battery jars. One jar was filled with water and covered by a piece of corrugated paper pierced with two holes; a stalk of alfalfa bearing pea aphids was inserted in one hole and a seedling corn plant bearing European corn borer eggs was placed in the other. The second jar, inverted over the first, contained a pair of adult spotted lady beetles. In another experiment a cardboard box, 12 in.×7 in.×4 in., was divided by a transverse partition and a pair of holes punctured through the bottom of each compartment. It was then placed over a pan of water and a leaf from a seedling corn plant and a stalk of alfalfa inserted through each pair of holes. The corn leaves each bore a European corn borer egg mass. The alfalfa stalk in one compartment bore pea aphids, the other did not. An adult spotted lady beetle was placed in each compartment and confined by a glass plate covering the box. In all these experiments, the feeding behavior of the beetles was observed for a minimum of 5 days.

Artificial Infestation of Plants.—European corn borer larvae were collected from fields during the fall and winter of 1957. These overwintered in an outdoor cage and were taken into the greenhouse at Newark in April. The emerging adults were introduced into the oviposition chamber referred to above (Briand 1929), in which eggs were deposited on waxed paper. The egg masses were cut from the paper and the individual eggs counted. They were then placed on the adhesive surface of a ½ in.×6 in. strip of plastic adhesive tape. Six or seven masses were placed in a row on this tape in such a manner that none of the adhesive surface was exposed to interfere with feeding. This strip was taped to the undersurface of a blade of corn in a field at Newark and was then covered by a 5 in.×2 in.×1 in. cage constructed from 16-mesh wire screen in such a way that it could be attached to the leaf beneath the eggs. An adult spotted lady beetle was placed in the cage, to remain there throughout the experiment. Each day the strip of tape holding the egg masses was removed and replaced by a freshly constructed one. Each strip was examined under the microscrope, upon return to the laboratory, to determine the number of eggs consumed. A maximum-minimum thermometer, the upper surface of which was shaded to simulate conditions existing beneath the leaf, was placed on a level with the cage and as close as possible to it. The temperature was recorded each day. Daily precipitation was determined by using an 8-in. rain guage.

Natural Feeding in Corn Fields—Two corn fields, about 5 miles apart, and both in the vicinity of potato fields heavily infested by first generation European corn borer, were selected in Kent County for these observations. One was located near Pearson's Corner and the other near Dover. As each European corn borer egg mass was located, the leaf bearing it was marked and a 3- by 5-inch index card was suspended under it by plastic strips (fig. 1.). The cards, waterproofed by dipping in hot paraffin, were coated on the upper surface with a thin layer of tanglefoot to catch the fecal pellets of any predators feeding on the egg masses. The fields were visited twice a week to hunt egg masses, to place cards and to examine cards previously placed. As soon as an egg mass, under observation, had hatched or disappeared, the card beneath it was removed and returned to the laboratory for examination. No more than three rows in either field were used in this study, since thus limiting the area facilitated return to all the marked leaves.

RESULTS.—*Bionomics of the Spotted Lady Beetle in Delaware.*—The adult is easily identified in the field (fig. 2). The ground color varies from rose to crimson. A pair of black spots appears on the thorax and 10 black spots on the elytra, 2 of which are centered between the elytra. The prothorax is approximately 5 mm. long by 3 mm. wide. The larva has a ground color of creamy yellow, a pair of cocoa brown spots on each thoracic segment, and mottled brown and yellow patches on all the abdominal segments except the fourth, which is almost entirely yellow.

It was found to overwinter as an adult either individually under the bark of the American sycamore, *Platanus occidentalis* L., under leaf sheaths of corn, or in aggregations at the base of trees and beneath rubbish. During the early spring it commonly was observed on the flowers of yellow rocket, *Barbarea vulgaris* R., and dandelion, *Taraxacum officinale* Weber. Later in the spring it established itself in clovers and alfalfa and during the summer months was found more or less abundantly on all crops in Delaware. The larvae, however, could not be found except in clover, alfalfa, and aphid-infested corn. The number of generations appeared to be from four to five a year.

The only parasite of the spotted lady beetle according to Thompson (1943) is a braconid, *Perilitus coccinellae* (Schrank) which attacks only the adult, as far as is known. During the course of this study observations were made upon the amount of parasitism in the field. At Pearson's Corner in the first week of July 191 adult lady beetles were observed on 700 plants; three of these were obviously parasitized as they bore the characteristic cocoon of *P. coccinellae* between their legs. This indicated about 1% parasitism. Later in the season it became apparent that a greater percentage were parasitized, although no actual counts were made. Only *P. coccinellae* adults were reared from this material (Cushman 1922).

Caged Feeding.—Observations on a caged adult of the spotted lady beetle indicate that it has a high predation

FIG. 1.—Cards suspended from corn leaves to collect fecula of the spotted lady beetle.

potential on eggs (table 1.). During the 13 days of this experiment 713 eggs were consumed, an average of nearly 60 a day, and it is probable that more would have been eaten had they been available. Neither temperature nor precipitation appeared to have any significant effects upon the number of eggs eaten. It was observed also, that the larger egg masses are seldom entirely consumed.

Food Preference.—In these studies it was found that this lady beetle would not feed on corn pollen, pea aphids, or European corn borer eggs when these were simply placed in the bottom of the cage. They did feed upon corn pollen when it was offered on a piece of a leaf from a corn plant that had reached the tassel stage. Also they fed freely on pea aphids when these were on alfalfa plants. At no time, however, did they feed upon corn borer egg masses located either on corn seedlings or on isolated leaves from corn seedlings. The beetles actually appeared to avoid contact with the corn seedlings.

Natural Feeding.—A total of 436 egg masses was located in the two fields in Kent County and the tanglefoot-coated cards placed beneath them. By comparing cards containing the fecula of the spotted lady beetle with those free of pellets, the percentage of egg masses fed on could be ascertained. The presence of at least one fecal pellet of the spotted lady beetle on a card was taken as

Table 1.—Amount of feeding by one adult *Coleomegilla Maculata* on European corn borer eggs in a leaf cage on sweet corn, Newark, Delaware, 1958.

DATE	NO. OF EGGS EATEN	TEMPERATURE (° C.)			PRECIPITATION (IN.)
		MAX.	MIN.	MEAN	
June 11	75	36	21	28.5	0.95
12	55	37	18	27.5	0
13	?[a]	38	21	29.5	0.01
14	33	33	10	21.5	0
15	31	25	10	17.5	0
16	44	29	10	19.5	0
17	75	30	8	19.0	0
18	63	31	11	21.0	0.21
19	45	32	15	23.5	0.21
20	83	21	15	18.0	0.05
21	65	22	13	17.5	1.3
22	78	29	11	20.0	0
23	66	32	11	21.5	0
Total	713				
Average	59.4				

[a] Two masses of eggs had hatched; 65 larvae were missing; 12 eggs were eaten.

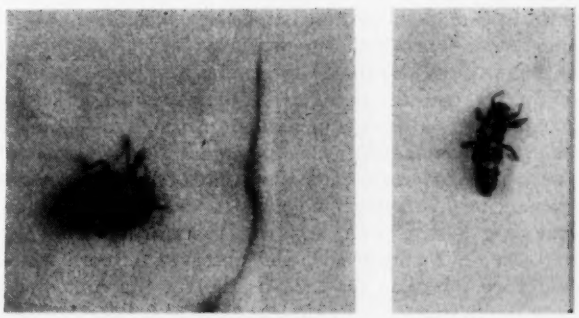

FIG. 2.—Adult and larva of the spotted lady beetle, *Coleomegilla Maculata* (De G.).

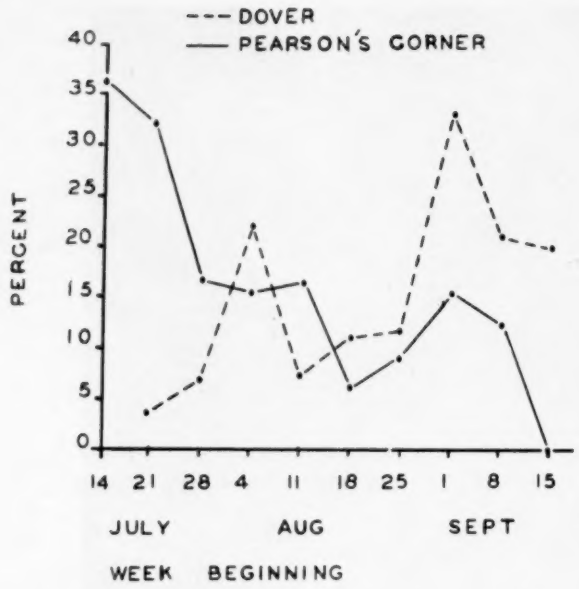

FIG. 3.—Percentage of European corn borer egg masses fed on by the spotted lady beetle in corn fields at Dover and Pearson's Corner during the summer of 1958.

a positive evidence of feeding. The ratio of these positive cards to those cards showing no evidence of fecula was compiled twice a week and the percentage of eggs fed on computed and averaged on a weekly basis. These percentages were taken for the fields under observation and plotted on a graph (fig. 3). On an average at Pearson's Corner and at Dover, of the tagged egg masses under observations, 16.5 and 15.3%, respectively, were fed on by this beetle.

DISCUSSION.—The method for determining the amount of feeding in the field originated from suggestions of S. W. Frost (1929). He states that the feculae of insects are a clear indication of their presence even after they have ceased feeding and have disappeared. Moreover, he indicated that the order and family can be determined by a critical examination of the color, texture, and size of the pellet. He further maintained that beetles, among others, discharge waste material soon after taking food because of their short digestive tract. Field observations of the fecal droppings of various insects tend to substantiate this.

Bearing this in mind, a critical examination of the previously undescribed fecula of the spotted lady beetle was made. When first dropped they are irregular, elongate, and light brown. They contain a variable amount of pinkish curdlike material at the terminals and may have other small irregular patches of this same material scattered over the surface (fig. 4-A). With age they turn almost black, but the pink-tinged areas remain. The size varies from 0.75 to 1.1 mm. long by 0.25 to 0.32 mm. wide. The pellet of the larva (fig. 4-B) is similar, both in color and size. The only fecal pellet observed on corn which might be mistaken for these is that of the convergent lady beetle (fig. 4-C), but in this case the pellet is marked with irregular patches of white curdlike material.

The number of fecal pellets found per card varied from one to nine, but most commonly there were three to five. The number of pellets could probably serve as an indicator of the relative number of eggs consumed in the mass but the developing of such an index was not considered during this work.

In figure 3 a high incidence of feeding at Pearson's Corner during the first week is indicated. This is followed by a rapid decline, a leveling off, another decline, a rise and then a sharp decline as borer-egg deposition finally subsided. The first decline is attributed to an increase in population of the corn leaf aphid and the second decline to the presence of pollen on the leaves. It has already been pointed out that both of these are highly favored foods of this beetle. The marked difference between the feeding curves of the two fields is in great part because of a difference in the stage of development of the plants. The corn at Pearson's Corner was in the pre-tassel stage when the first-generation borer adults commenced ovipositing, while the corn in the field at Dover was farther advanced, being well tasseled and with ears starting to form.

Another major difference between the two fields was that the population of the spotted lady beetle at Pearson's Corner was higher (29 per 100 plants) than at Dover (3 per 100 plants) during the first few weeks of observation. As the season progressed, however, the population of these beetles tended to increase at Dover and to decrease at Pearson's Corner. This fluctuation in density of the spotted lady beetle in the two fields presents a problem that was not resolved. It seemed evident from the food preference tests that the presence of the egg masses on the corn was not the attractant factor. The stage of the corn, on the other hand, appeared to be an attractant stimulus. The European corn borer ordinarily is more attracted to corn in the pre-tassel stage for oviposition than to the older corn. In this case, however,

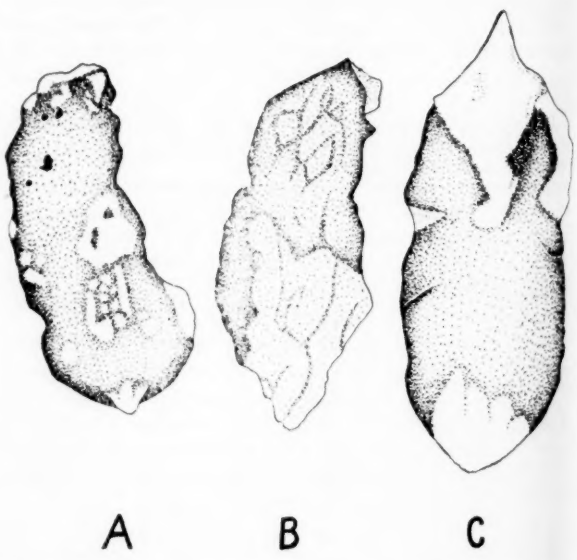

FIG. 4.—Fecula of lady beetles. A. Pellet of spotted lady beetle *Coleomegilla maculata* (De G.), adult. B. Pellet of spotted lady beetle larva. C. Pellet of convergent lady beetle, *Hippodamia convergens* (Guer.), adult.

they oviposited heavily on both the younger and older plants. This fact would indicate that the corn borer is not overly particular about its egg-laying site when its population is high, as it was in this case, and there is an acceptable host plant available. On the other hand, the spotted lady beetle appears to be more particular, and is attracted more to pre-tassel corn than to older plants. Thus the simultaneous appearance of borer eggs and spotted lady beetles on corn results from this beetle's not being attracted to corn until it has attained the height at which it is most attractive for corn borer oviposition.

The amount of feeding on corn borer eggs by the spotted lady beetle was less than anticipated; only about 16% of the egg masses were fed upon. The percentage of eggs actually consumed could not be determined by this method for, except in rare instances, the larger egg masses were not entirely consumed. The beetles have such a restless feeding habit that slight disturbances cause them to cease feeding and to fly away, seldom to return to the same egg mass.

Other species of insects were occasionally observed feeding on the egg masses. These were larvae of chrysopids and an adult *Orius* sp. Adult chrysopids were not observed feeding on corn borer eggs but their eggs were observed on corn leaves early in the spring and the adults were very common all summer. A considerable number of the tanglefoot-coated cards contained their feculae, which are distinguished by a dark color and cylindrical shape. This fact suggests that they may feed on corn borer eggs, but such activity was never observed.

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The Description of Insect Numbers[1]

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ABSTRACT

In economic entomology, particularly forest entomology, the description of insect numbers in absolute terms may be misleading. The effect of an individual on its host varies tremendously between species and this gives rise to unwarranted comparisons within current systems. The insect-host relationship between *Recurvaria starki* Freeman and *Pinus contorta* Dougl. is reviewed and used as an example in the erection of a three-class system for the description of insect numbers based on the effect of the insect on its host.

At the present time, a number of terms are used to describe levels of insect abundance. Through repeated usage these gain general acceptance and a limited or standard meaning when applied to single insect species. However, because the same terms are applied to populations of many different insects, confusion exists with respect to their exact meanings. With the ever increasing interest in critical sampling and interpretation of sampling results in terms of population dynamics (Morris 1955), a description of insect numbers based on biological grounds would be of value.

TERMS USED.—In the literature of economic entomology, "epidemic" and its corollaries are used to describe a population of insects which, for some reason is considered to be of adverse economic effect. "Endemic" is used to describe a population which is of no current economic interest. "Endemic" is also used to describe an organism which is native to the area concerned (Benton & Werner

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1958). Both terms are used in public health and other fields and both suffer from imprecision and overlapping meaning when applied to the results of numerical sampling. Indeed, their utility may to some extent depend on this imprecision when the terms are used in general discussion.

In the discussion of precise data, the concept of "density" is frequently used. In its strict sense, "density" refers to the average number of objects in a unit of space. By custom however, the term has come to mean the number of animals in relation to the "available" space. The use of the term "density" in the wider sense is well established and there seems to be little objection to it. However, as Robertson & Sang (1944) have pointed out, the substrate is always to some extent heterogeneous and there are difficulties in the use of any measurement of density which depends on the use of absolute units of space. In view of such difficulties, the more neutral term "crowding" has been suggested by these authors but it seems difficult to interpret and to standardize.

A further difficulty in the development of a terminology lies in the requirement that the same terms have similar meanings when applied to different species of insects. Certainly, it is impossible to regard the usual term "epidemic" as having a standard numerical or biological meaning. For instance, a population of 1,000 adult *Pissodes strobi* Peck per acre of white pine reproduction would be regarded as "epidemic." On the other hand, a population of 1,000 *Pineus strobi* Htg. per acre of white pine reproduction would be regarded as "endemic" if indeed it could be detected at all.

Recognition of this situation has repeatedly led to attempts to express insect population data in general terms. The most frequent attempts have been toward the development of frequency functions with respect to some unit of the substrate (Clarke 1954) ("density") or on an area basis (Henson 1954) ("absolute density"). Such expressions do not describe the differing biological implications of individuals of different species from the host standpoint. As the main interest in economic entomology is most often in the effect which populations have on their hosts, it would seem advisable to attempt to describe insect numbers in terms of this effect.

INSECT EFFECT ON HOST.—Two possible criteria of insect effect on the host appear to warrant consideration. First, it should be possible to set limits of economically tolerable insect damage. If such limits were set, insect numbers could be expressed with respect to the numbers required to produce minimum intolerable damage. However, because economic values change, such a system would be unstable and unrealistic.

The second possible criterion would be the biological balance between the productivity of the host and the attrition of the host by the insect. Allee *et al.* (1949, p. 245) point out that "In a sense, animals as a whole are broadly symbiotic in their relations with the plant kingdom . . . feeding on their surplus without doing them vital harm." This must be true "in a sense" as evidenced by the continued co-existence of plants and animals. However, at least on a local level, vital harm does come to plants as the result of insects' activities.

As Clark (1927) pointed out some years ago, trees produce more leaves and more tissues of other sorts than are necessary for growth, survival and reproduction under average conditions. If insect attrition is limited to the comsumption of this "surplus," no vital harm will be done to the hosts. An insect population which confined its depredations to the consumption of the surplus would be of importance in economic entomology only as a possible nucleus of a larger population or because of its nuisance value.

The concept involved in the definition of this level of insect activity bears some relationship to the "carrying capacity," a term used in grassland ecology and by Allee *et al.* (1949, p. 707) with reference to deer. " . . . The normal carrying capacity . . . (a) number (which) does not damage the plant forage as do excessive population eruptions." Clarke (1954) also used the same term and defines it as "the maximum standing crop that can *maintain itself indefinitely* in an area . . . " (italics ours).

The most useful datum for our present purposes is that level of population which just balances the biological surplus productivity of the host. This population level would be species specific and also a specific characteristic of the stand under investigation. For the present purposes, the biological productivity of the host is considered to be just exceeded at the minimum level of insect attrition which results in the reduction of growth.

The studies of Mott *et al.* (1957) on the effects of insect defoliation on balsam fir, tamarack, and lodgepole pine give reasonably precise techniques for assessing the influence of defoliation on radial increment. From these and other studies, it is possible to calculate maximum defoliation levels which do not affect the growth of host trees on a given site.

Stark & Cook (1957) have calculated the amount of defoliation that would be caused by a series of populations of a lodgepole needle miner (*Recurvaria starki* Freeman) on lodgepole pine over a number of years. The relationship between rate of defoliation, time and population is illustrated by the family of curves presented in figure 1.

In this diagram, insect densities are expressed in terms of numbers of insects per branch tip, a biological unit of the substrate rather than a unit of absolute area. From the graph, one may conclude that a population of 70 or more larvae per tip will defoliate the trees completely if

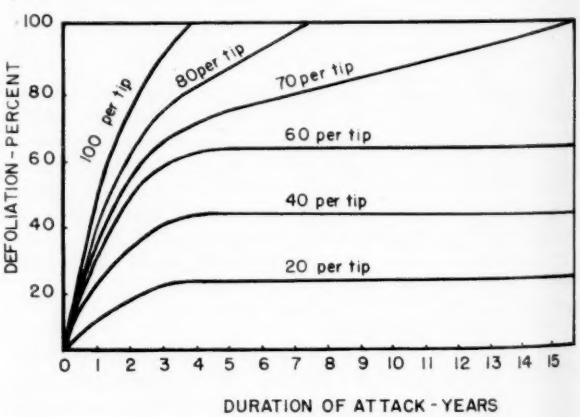

FIG 1.—Theoretical defoliation of lodgepole pine with different intensities of needle miner attack. (Redrawn from Stark and Cook 1957.)

the population remains constant and if the same amount of foliage is produced each year. Populations of 60 per tip or less will continue to effect a constant level of defoliation as long as the production of foliage is constant. At some level between 60 and 70 insects per tip, there is a balance between the production of needles by the trees and the depredations of the insects.

Needless to say, the trees could not continue to produce a supply of needles year after year under such conditions. A population which would just balance the physiological over-production of needles in this system would be considerably lower than 60 per tip. In lodgepole pine, growth is significantly reduced by 40% defoliation. No effect is detected at 10% defoliation (Stark & Cook 1957). Radial increment is noticeably affected at 40 to 50% defoliation (Mott *et al.* 1957). The largest population of needle miner which would not deplete the resources of the trees would thus be about 10 larvae per tip. Above this level, a smaller population would produce the same amount of defoliation after some years because the capacity of the tree to produce the same annual crop of needles would be compromised.

CATEGORIES OF POPULATIONS.—On the basis of these findings, we may distinguish three general categories of populations:

1. Populations that do not utilize the entire excess biological productivity of the host. Such populations may be called "*Tolerable.*"

2. Populations that utilize more than the excess biological productivity of the host but less than the total productivity. Such populations may be called "*Critical.*" They imply a temporary situation since they must affect the host through the reduction of the annual output. If such populations are maintained over a period of time, the host's productivity will decline.

3. Populations that are depleting the host at a rate greater than the current rate of production. Such populations reduce the host capital. They may persist for years but, ultimately, attrition destroys the host if the populations remain constant. These populations may be called "*Intolerable.*" They represent self-destructive situations in which the death of the host is unavoidable if the populations remain constant. A special case of intolerable population levels is experienced when a population reaches a level so high that the substrate is exhausted in less than the course of a single generation. In such a case, the insects starve. In some special cases such as that of defoliators of broad leaf trees, the consequences of such extreme populations from the host standpoint are relatively favorable. Insect populations are reduced by starvation while the host can still recover from the effects of a single defoliation.

The terms are suggested because they seem to relate to the position of the substrate with respect to the insect population. Certainly a population which was consuming only the biological excess would be tolerable from the host's viewpoint. A consumption which exceeded the substrate's excess would be critical in any case and one which exceeded the total output would be intolerable.

Tolerable Populations.—The relative levels of lodgepole needle miner populations which would correspond to these various categories may be derived from the previously cited studies (Mott *et al.* 1957, Stark & Cook 1957). Healthy trees produce about 75 needles each year on each growing terminal of the upper crown. Since sampling is based on branch ends each of which contains the growth of 5 years, the average size of the substrate unit is 375 needles. A "tolerable" population would be about 10 larvae per tip or fewer. Such a population has been found to effect about 10% defoliation over a period of years. No effect on the normal growth of the trees can be demonstrated for such populations.

"*Critical*" *populations* would run from 10 to 60 larvae per tip. At these levels, a constant defoliation characteristic of each population is reached and maintained. In time, however, the productivity of the tree is reduced by the attrition. When this happens, the size of the population which would maintain a constant defoliation would be reduced. A constant population would have more and more effect on the tree until its depredations exceeded the reduced productivity of the tree.

"*Intolerable*" *populations* would run above 60 larvae per tip. At these levels, the annual depredations of the insects would exceed the total productivity of the host. At a population of 150 per tip, the tree's foliage would be exhausted before the development of the current population was completed. If a stand had sustained "critical" or "intolerable" populations in the years leading up to the development of the very high populations, fewer than 150 larvae per tip might starve by exhausting the reduced reserves of the previously damaged trees.

RELATIONSHIP BETWEEN CATEGORIES.—A three dimensional model which illustrates the relationship between the categories of populations may be constructed. Such a model is presented in graphic form in figure 2. The tip size is shown to vary between 0 and 375 needles on the X axis. The number of larvae per tip between 0 and 60 is shown on the Y axis. Eight generations (16 years) are shown on the Z axis.

The upper limit of tolerable populations is a surface extending from the XY origin to X_{375}, Y_{10}. As the system represented is stable, there is no change in time and the surface is flat. The upper limit of critical populations is a surface which intersects the Z_0 plane along a line extending from the origin to X_{375}, Y_{60}. As time increases, the Y value for X_{375} decreases as an expression of the fact that these populations, inasmuch as they exceed the excess productivity of the tree would, in time, compromise the tree's annual production.

This model is characteristic of the stands for which it was constructed and certainly for the insect-host system which it represents. It is predictive only in the sense that it suggests the outcome of continued defoliation by constant populations. The nature of population dynamics in this insect has already been outlined (Stark 1956) and stable populations would certainly be unusual. The most basic parts of the model do have, nevertheless, some generality. These considerations pertain primarily to forest insect populations and may not be generally applicable to fruit, field crop, or stored products insects.

Categories of populations which would have quite general application could be erected with relation to the productivity of whole stands (on the basis of a unit of area). Such a model could be used to describe populations of defoliators by an extension of the type of calculations already presented. It could also be used to describe

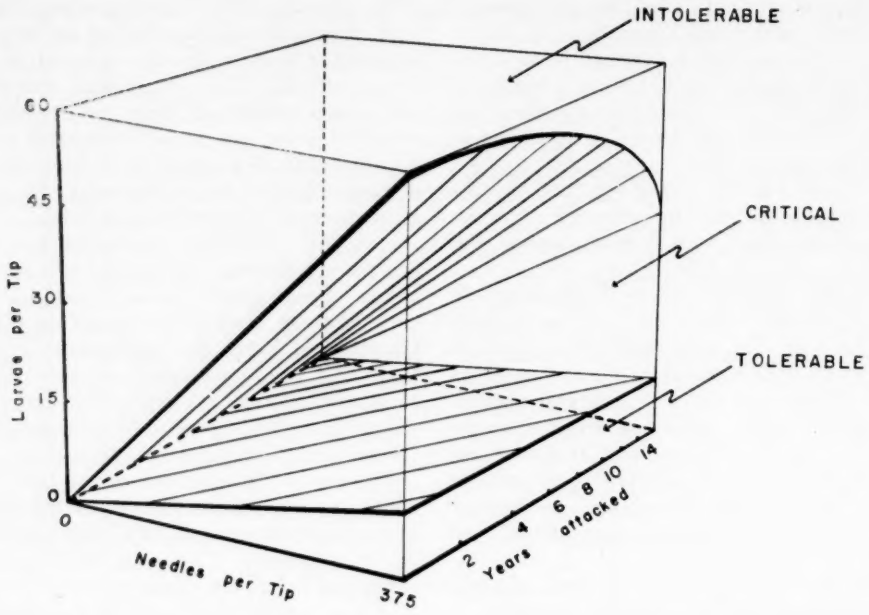

FIG. 2.—Diagram of the relation between three different criteria of population.

populations of insects like bark beetles which cause isolated individual mortality by virtue of the fact that the expression of substrate size would be in units which comprised many individuals. By the same token, the model could be used to describe populations of insects such as terminal weevils which cause critical deformation of individual trees. In this case, the unit of substrate size would express the number and size of undamaged host individuals.

If studies of the population dynamics of an insect made possible a predictive expression of the numbers-time relationship, such a finding could be applied directly within the frame of the present model. If the resultant surface representing an actual population intersected the boundaries of a population category, the implications from the standpoint of stand management would be immediate and powerful.

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Chemical Control of the Onion Maggot in Onions Grown from Seed in Various Types of Soil in Northwestern North America in 1955 and 1956[1]

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Abstract

In duplicated experiments in 1955 in British Columbia, Idaho, Oregon, and Washington, the chlorinated hydrocarbons, dieldrin, heptachlor, and isodrin, however applied, gave economic control of the onion maggot (*Hylemya antiqua* (Meig.)) under irrigated conditions in the interior of British Columbia and Idaho, but malathion allowed as much damage as in the untreated plots. In western Washington and western Oregon, neither dieldrin nor heptachlor gave economic control, but isodrin and malathion warranted further investigation. In plots in which formaldehyde was dripped into the furrow against onion smut in Oregon muck soil, fewer seedlings emerged than in those for which the seed was treated with thiram. At three localities, where emulsible insecticides were applied as drenches to the seed furrow, fewer seedlings emerged than where wettable powders and dusts were applied. At the fourth site this difference was not so apparent. In similar experiments in 1956 furrow and seed treatments, with dieldrin, endrin, and heptachlor allowed 0.8% maggot damage in onions in a sandy clay loam and 5.1% in a peat soil in the interior of British Columbia, but malathion gave no more protection than plots left untreated. In muck soil in western Oregon, damage in the untreated plots was 47.3%, but malathion allowed only 4.2 to 7.7% loss and endrin 5.7 to 14.4%. The results indicate that soil types or conditions influence the effectiveness of insecticides markedly. In western Washington and in Idaho in 1956, none of the treatments were satisfactory, damage ranging from 75 to 100% and from 38 to 71% respectively; these results suggest at least that strains of the maggot resistant to these insecticides were developing.

In the large onion-growing areas in North America, New York, Connecticut, Michigan, Wisconsin, Minnesota, Idaho, Oregon, Washington, Quebec, Ontario, Manitoba and British Columbia, severe infestations of the onion maggot, *Hylemya antiqua* (Meig.), have occurred, the crop losses totaling millions of dollars. Since 1946, various degrees of economic control have been obtained with chlorinated hydrocarbon insecticides (McLeod 1946, Rawlins & Newhall 1950, Finlayson & Handford 1954, Peterson & Noetzel 1954, Tozloski 1954, Finlayson 1957, Shirck 1957). However, in January 1955, at the 14th Annual Northwest Vegetable Insect Conference at Portland, Oregon, discussion of the onion maggot problem suggested that the effectiveness of the insecticides was decreasing in various districts of Washington and Oregon. It was pointed out that before 1953, in coastal Oregon, DDT and chlordane applied as drenches to the furrow with the seed were satisfactory. In Washington aldrin, heptachlor, chlordane and dieldrin were satisfactory in furrow, seed, and surface treatments until about the same period. In British Columbia and Idaho seed treatment with dieldrin and heptachlor were effective at least until the initiation of this investigation.

A common experiment was therefore designed on the basis of the most effective insecticides and methods of application known in each of the four Pacific northwest areas: British Columbia, Idaho, Oregon, and Washington. The objectives were: (1) to investigate the factors causing different results in the various areas and (2) to develop a common method of preventing damage by the maggot in the various soil types and under different climatic conditions. This is a report of the experiments conducted in 1955 and 1956.

Materials and Methods.—In 1955 the investigation was conducted at four localities: Kamloops, British Columbia (sandy clay loam); Parma, Idaho (clay loam); Puyallup, Washington (clay loam); and Lake Labish, Oregon (muck soil). The experiment involved 16 insecticide and 1 fungicide treatment, and 1 untreated check, each replicated 5 times in a split-plot design. Half of each plot for the insecticide and fungicide treatments was treated with thiram at 2.0 oz. of toxicant per pound of seed for smut control (Larson & Walker 1953). The other half was left untreated except at Lake Labish, where formaldehyde was applied at 1 pint of solution per 20 feet of row; the solution was prepared by mixing 1 pint of 37 to 40% formalin in 16 gal. of water. United States measurements were used in all cases. The untreated check did not receive either of the fungicide treatments.

Each half-plot consisted of one row of onions 20 feet long. Seed was sown at 3.5 lb. per acre, or 0.96 grams per 20 feet. This amount was calculated for rows spaced 16 inches apart. All seeding was done with a rod-row, multiple-gear, V-belt seeder. The variety White Sweet Spanish was grown at Parma and Puyallup, and Oregon Yellow Danvers at Lake Labish and Kamloops.

Dieldrin, heptachlor, isodrin, and malathion were applied as seed and as furrow treatments. Table 1 lists the formulations and the methods and rates of application.

Seed treatments were applied by wetting weighed seed samples with water and mixing measured amounts of insecticide and/or fungicide with the dampened seed until uniform coatings were achieved.

Furrow treatments were applied in two ways: (1) as

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Table 1.—Materials and methods and rates of application used in onion maggot experiments in the Pacific Northwest in 1955 and 1956.[a]

Material	Method	Rate[b] 1955	Rate[b] 1956
Dieldrin			
50% (w.p.)	seed	0.5	0.5
	seed	—	1.0
	furrow	16.0	16.0
	furrow	—	32.0
1.5% (dust)	furrow	16.0	—
15% (emulsion)	furrow	32.0	—
Heptachlor			
25% (w.p.)	seed	0.5	0.5
	seed	—	1.0
	furrow	16.0	16.0
	furrow	—	32.0
2.5% (dust)	furrow	16.0	—
20% (emulsion)	furrow	32.0	—
Isodrin			
25% (w.p.)	seed	0.5	—
	furrow	16.0	—
2.0% (dust)	furrow	16.0	—
16% (emulsion)	furrow	32.0	—
Endrin			
25% (w.p.)	seed	—	0.5
	seed	—	1.0
	furrow	—	16.0
	furrow	—	32.0
Malathion			
25% (w.p.)	seed	0.5	0.5
	seed	—	1.0
	furrow	16.0	16.0
	furrow	—	32.0
4% (dust)	furrow	16.0	—
57% (emulsion)	furrow	32.0	—
Formaldehyde[c]	furrow	1 pt.	—
Thiram (SFX)[c]			
75% (w.p.)	seed	2.0	2.0

[a] The insecticides used at each locality came from the same mixture of each formulation.

[b] Seed treatments, ounces of toxicant per pound of seed; furrow treatments, ounces per acre.

[c] See "Materials and Methods" for a discussion of the thiram and formaldehyde treatments.

dry applications with the seed and (2) as drenches. For a dry application, a dust or a wettable powder was spread uniformly over the seed in the V-belt of the seeder and sown simultaneously with the seed. For a drench application, the solution was prepared by mixing the emulsible concentrate in 1 pint of water, or in formaldehyde solution in Oregon. The solution was gravity-fed from a rubber tube connected to a bottle suspended from the handle-bar of the seeder. The liquid spilled over the seed in the open trench just before the boot closed the furrow.

Phytotoxicity of the chemicals was assessed in two ways. First, the effects of the chemicals on germination were appraised by counting the seedlings in each plot. This count was made about 20 days after approximately 50% of the seedlings were in the loop stage. Secondly, the aerial parts of the onions were observed for phytotoxic symptoms throughout the period.

Damage by the maggots was appraised at approximately weekly intervals until the end of June and at 10-day intervals from then until harvest. Affected plants, indicated by wilting, were pulled, examined to confirm the damage, and returned to the surface of the ground in the row where they had been growing. This practice allowed immature stages of the maggot to return to the soil. Percentage damage was calculated from the total numbers of damaged plants and seedlings. At harvest all remaining onions were pulled and examined for maggot injury. The sound onions were left for a week to cure, after which the yield was determined by weight in ounces. Data were collected from the full 20 feet of row.

In 1956 several modifications were made to the above procedures. The investigation was conducted at five locations: Kelowna, British Columbia (two sets of plots, one in peat soil and one in sandy clay loam); Parma, Idaho (clay loam); Lake Labish, Oregon (much soil); and Puyallup, Washington (clay loam). The plots were arranged in randomized blocks, in five replications. Each replicate had 16 insecticide treatments, 1 fungicide treatment and 1 untreated check. Thiram was used for all plots except the checks. Each plot consisted of three rows 20 feet long. All records were made from the central row of each plot. Rate and method of seeding and varieties of onions were the same as in 1955.

Wettable powders of the insecticides (endrin in place of isodrin because the manufacturer discontinued production) were applied as seed treatments and as dry furrow treatments. Formaldehyde was not used. Table 1 lists the formulations and methods and rates of application. Maggot damage was assessed as in 1955 except that the damaged onions were dropped between instead of in the rows. Phytotoxicity and yield were appraised as in 1955.

Soil samples were taken from all sites in 1956 and analysed.

To determine the effects of the seed treatment on germination, 4 replicates of 25 seeds for each variety and for each treatment and rate were placed in closed petri dishes on 2 thicknesses of moist filter paper in a rearing cabinet at 75° F. and 70% relative humidity. Water was added when needed.

Data on germination, emergence, damage, and yield were examined by analysis of variance and the multiple range test (Duncan 1955). Differences were considered significant when they exceeded the amount necessary at the 5% level.

RESULTS IN 1955.—Data for the 1955 experiments are presented in figure 1. There were no visible signs of phytotoxicity from any of the treatments. Significant differences between treatments and treatments and checks are dealt with by localities.

Puyallup, Washington (fig. 1A).—Thiram-treated plots had a significantly greater number of seedlings and a higher percentage of maggot damage than those left untreated. Three isodrin treatments, the seed and the two dry furrow treatments, produced the greatest number of seedlings; those of the latter two were significantly greater than those of the untreated check. Drench applications caused a reduction in the number of seedlings. The number of seedlings in the plots treated with isodrin by the drench method was significantly lower than those for all other treatments. Damage was heavy. Only six treatments allowed significantly less damage than the un-

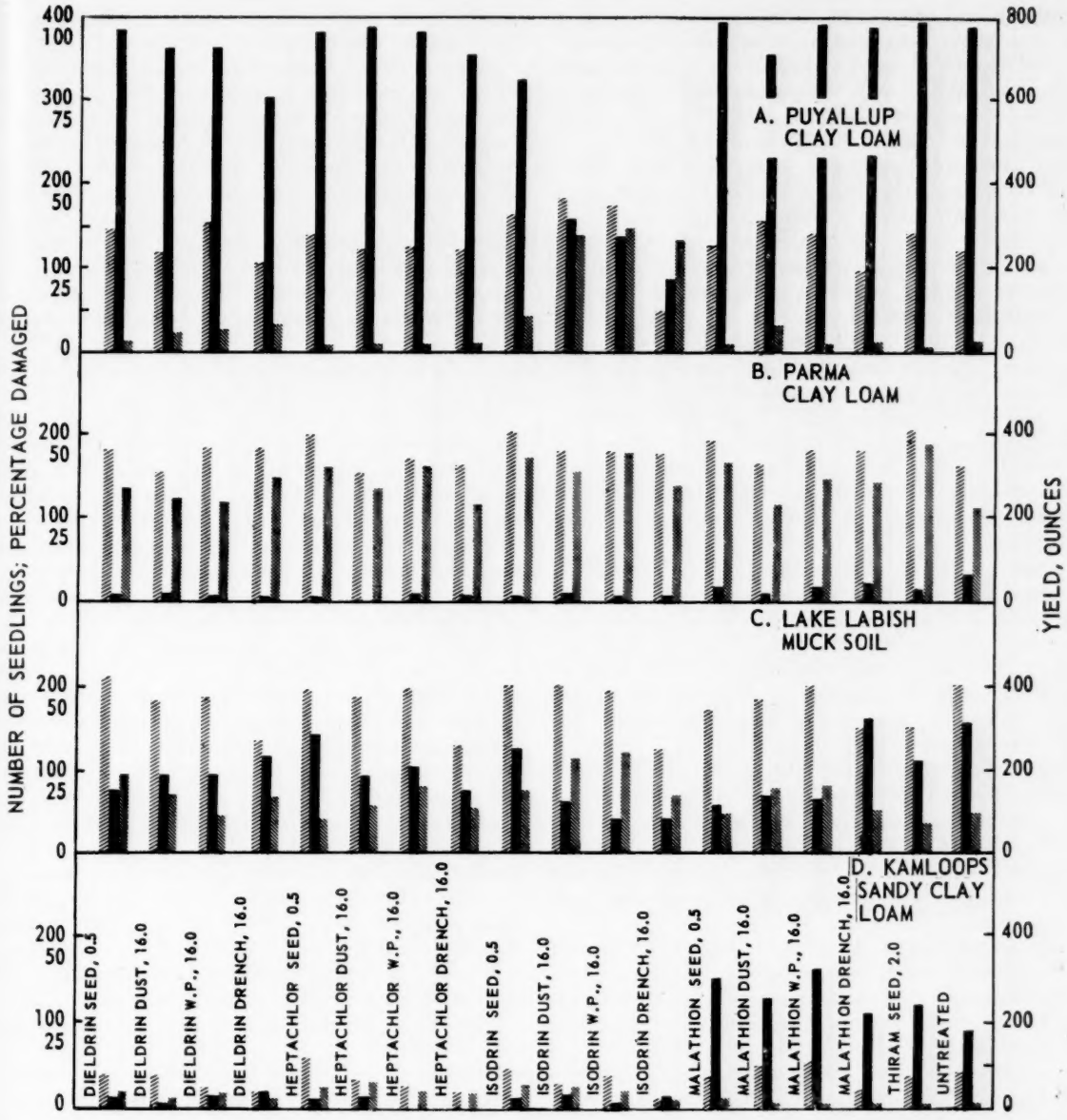

FIG. 1.—Average numbers of seedlings (light hatching), percentage damage (solid bar), and yield of undamaged onions (dark hatching) per 20 feet of row after various treatments against the onion maggot in 1955. (All seed used in the insecticide treatments was treated with thiram.)

treated checks, and of these only the isodrin drench treatment could be considered to be approaching the level necessary for commercial control. Yields, except for the isodrin furrow treatments, were no better than that of the untreated check.

Parma, Idaho (fig. 1B).—The number of seedlings varied widely, but only in the plots treated with dieldrin dust were there significantly fewer than in the untreated plots. The number of seedlings was significantly higher where thiram was used than where it was not. Although the maggot infestation was not severe, 5.9% damage in the check, four treatments, isodrin drench and wettable powder furrow treatments and dieldrin wettable powder and dust furrow treatments, allowed significantly less damage than the untreated check. The yield of marketable onions ranged from 200.8 to 331.2 ounces but none differed significantly from the check.

Lake Labish, Oregon (fig. 1C).—The number of seedlings in the check plot was significantly greater than that of any other treatment. Thiram-treated plots produced significantly more seedlings than those receiving formaldehyde. The furrow drench treatments had significantly fewer seedlings than those treated by the other methods. Damage ranged from 10.4 to 48.2%. Four treatments, three isodrin furrow treatments and the dieldrin seed treatment, allowed significantly less damage than the check. Yields varied greatly; only those for the four isodrin treatments were significantly greater than the checks.

Kamloops, British Columbia (fig. 1D).—The numbers

of seedlings that emerged at this site were much smaller than at the other three localities. The drench treatments had significantly fewer seedlings than the untreated check. None of the other treatments had significantly more or less seedlings than the check. The percentage damage ranged from 0 to 50.4%. The chlorinated-hydrocarbon treatments, regardless of method, gave significantly more protection than the malathion treatments which did not differ significantly from the untreated checks. Yields were reduced but those recorded for isodrin and heptachlor seed treatments and the furrow treatments with dust, were significantly higher than that produced in the untreated plots.

RESULTS IN 1956.—Tests in the laboratory showed that under optimum conditions percentage germination of the seed was not significantly altered after the seed had been treated with dieldrin, endrin, heptachlor and malathion. Partial soil analyses (table 2) suggested no reasons for the differences in percentage damage in the control of the onion maggot at the various sites. Data for the experiments are shown in figure 2 and are reported below by locality.

Parma, Idaho (fig. 2A).—There were no significant differences between any of the treatments in the numbers of seedlings and in percentage damage at mid-season.[8] However, by harvest damage ranged from 38.3 to 70.9%. Only two treatments, endrin seed and furrow treatments had significantly less damage than the checks. There were no differences in the yields between treatments nor between treatments and the check.

Puyallup, Washington (fig. 2B).—Although the number of seedlings ranged from 117.6 to 160.4, damage was so severe at this site that by harvest only two treatments had more than 3 ounces of marketable onions. The two treatments, endrin furrow treatments, allowed 75.5 and 89.6% damage. The remaining treatments had more than 99% damage.

Lake Labish, Oregon (Fig. 2C).—Endrin treatments had significantly more seedlings per plot than the check or the thiram treatment. At midseason all treatments, except the low rate of heptachlor applied to the furrow, had significantly less damage than the checks. At this time all treatments with endrin, dieldrin and malathion had at least 30% less damage than the checks. At harvest only five treatments, the four malathion treatments and the endrin furrow treatment at 32 ounces, had less than 10% damage. None differed significantly from the other and all allowed significantly less damage than the check. There were no significant differences among the yields for any of the treatments, the range of which was from 200.8 to 365.4 ounces from 20 feet of row.

Kelowna, British Columbia (sandy clay loam: fig. 2D).—Only the 0.5-ounce endrin seed-treated plots had significantly more seedlings than the check. However, the plots of the endrin treatments produced significantly more seedlings than any of the other three insecticides. Maggot damage at midseason was not severe, only 16.6% in the untreated check. All treatments with the chlorinated hydrocarbons and malathion at 32 ounces allowed significantly less damage than the checks, but did not differ significantly from each other. At harvest the damage in the untreated and thiram-treated plots was 26.7 and 31.4% respectively. Seed treatments of dieldrin at 1.0 ounce and endrin at 0.5 ounce produced significantly higher yields of onions than that of the untreated check.

Kelowna, British Columbia (peat soil: fig. 2E).—With the exception of dieldrin at 0.5 ounce, plots treated with the chlorinated hydrocarbons had significantly greater numbers of seedlings than that of the untreated check. Damage at midseason ranged from 0.7 to 80.1%. All treatments with endrin, dieldrin, and heptachlor and the malathion furrow treatment at 32 ounces allowed significantly less maggot damage than all other treatments. At harvest the same condition persisted. Malathion seed treatment at 0.5 ounce permitted significantly more damage than the untreated check both at midseason and at harvest. The yields from the plots treated with the chlorinated hydrocarbons, except those from dieldrin applied at 0.5 ounce differed significantly from those produced in all other treatments and from that of the untreated check.

DISCUSSION.—The results from the 2 years' experiments showed that effective and economic control of onion maggots attacking onions grown from seed is still possible with malathion and endrin in the coastal Oregon area, and chlorinated hydrocarbons in the interior of British Columbia. The insecticides did not give commercial control in the areas tested in Washington and Idaho. An insecticide that allows more than 20 to 25% damage by the onion maggot is considered of no value in commercial control.

Seedling Emergence and Phytotoxicity.—Phytotoxic effects similar to those reported by Finlayson (1952, 1957) were not evident for any of the treatments. At three locations seedling emergence was retarded by the application of emulsions as a drench to the seed in the furrow. This was evident whether the seed was treated with thiram or not. Finlayson & Handford (1954) reported similar conditions in the interior of British Columbia in sandy clay loam using emulsions of aldrin, chlordane, DDT, and dieldrin. In both cases it is suspected that the solvent or solvents used in the emulsible concentrate are the factors hampering germination. In addition to a reduction in the number of seedlings caused by emulsions, fewer seedlings emerged in the plots treated with formaldehyde. Formaldehyde applied to dry soil has been known to cause reduced germination since it was first used in smut control some 50 years ago (Larson & Walker 1953). Thiram and endrin permitted the emergence of

Table 2.—Components of soils in which onion maggot experiments were conducted in 1956.

Soil Factor	British Columbia		Idaho	Oregon	Washington
	Sandy Clay Loam	Peat Soil	Clay Loam	Muck Soil	Clay Loam
pH	6.5	6.6	6.8	5.3	5.7
Organic matter (%)	3.22	89.14	0.85	22.63	2.76
Calcium (meq./100 gm.)	10.27	36.03	8.85	12.69	4.23
Magnesium (meq./100 gm.)	2.66	10.27	2.17	2.30	0.96
Potassium (meq./100 gm.)	4.35	10.14	4.06	13.63	4.35
Exchange capacity (meq./100 gm.)	21.64	148.71	19.81	85.04	18.03
Percentage base saturation	79.88	37.95	76.12	33.65	79.88
Conductivity (millimhos)	16.0	16.0	8.0	13.3	8.0

[8] A change in personnel (D. R. Scott succeeded A. J. Waltz) did not permit counts to be made in the early part of the summer. These are necessary to determine the number of seedlings and first generation maggot damage.

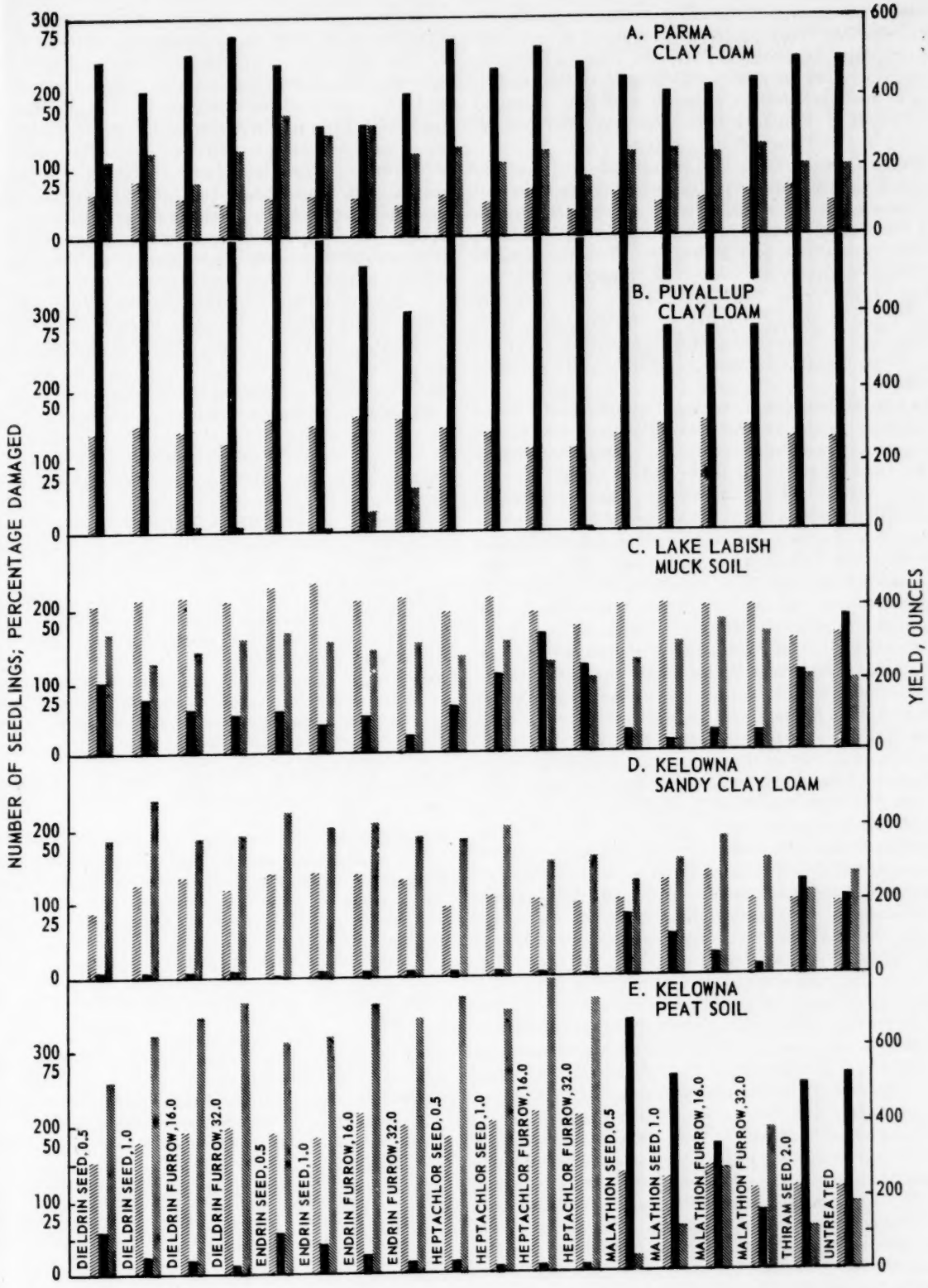

FIG. 2.—Average numbers of seedlings (light hatching), percentage damage (solid bar), and yield of undamaged onions (dark hatching) per 20 feet of row after various treatments against the onion maggot in 1956. (All seed used in the insecticide treatments was treated with thiram.)

greater numbers of seedlings. Thiram as a fungicide protects seedlings from damping-off and other seedlings diseases. Although the action of endrin is unknown to the authors, Richardson (1957, 1959) reported that endrin reduced root rot infection in barley seedlings, decreased early blight development in tomatoes, and reduced the severity of Fusarium wilt in tomatoes.

Maggot Damage.—The 1955 results indicated that isodrin and malathion would protect onions grown from seed against attacks by the onion maggot in coastal Oregon and Washington, and that the chlorinated hydrocarbons, dieldrin, heptachlor, and isodrin, would do the same in the interior. In trying to evaluate these findings, soil conditions and types of soil as well as soil properties were considered. In the 1956 trials soil analyses were completed on soil types from all locations with the hope that the answer for discrepancies in the results would be explained.

In coastal Oregon in a muck soil with a pH of 5.3 and an organic content of 22.6% the malathion and endrin treatments gave effective control, with malathion slightly better than the endrin. Dieldrin and heptachlor were, as in 1955, mediocre or not much better than the untreated check. In contrast to this in the interior of British Columbia, in a sandy clay loam with a pH of 6.5 and organic content of 3.2%, and in a peat soil with pH of 6.6 and organic content of 89.1%, 12 different treatments with dieldrin, endrin, and heptachlor were far superior to the malathion treatments. Moreover, the malathion treatment which allowed least damage in Oregon had the same amount of damage as the check, and a lighter application which allowed only 7.8% damage in Oregon had 84.3% in the peat soil in British Columbia. With these reversals in maggot damage it appeared that soil conditions might affect the efficiency of the insecticides.

In view of the above results it was not unreasonable to assume that the results from Washington and Idaho would follow a similar trend. Although the soil types were the same, differences in pH were similar to those of Oregon and British Columbia. However, when the results on control of maggot damage were assessed this assumption was found to be wrong. The percentage damage in Washington reached 100% for 10 of the 16 insecticidal treatments. Only two treatments had less than 99% damage; these were endrin treatments which had 75.5 and 89.6% damage. Although the results in Idaho were not quite so disappointing, the trend was the same. Heptachlor treatments which had shown some promise in 1955 had higher percentage damage than the untreated check. Again only the endrin treatments showed any degree of control and this was far from satisfactory.

In the fall of 1956 reports from the various onion districts in Washington, Idaho and eastern Oregon indicated that control recommendations were no longer satisfactory for these areas. Chlordane treatments in the Walla Walla area in Washington, which had been used with good results for several years, did not give the required protection. In 1957 some farmers in Idaho and eastern Oregon, after experiencing heavy onion maggot population increases in the fall of 1956, lost high percentages of their seedlings in the spring.

Similar conditions developed in Michigan. Guyer (1957) reported that an increased population in the fall of 1956 was followed by failures of recommended measures which previously had been economically sound. He further stated that the condition was not localized but that the pattern persisted throughout the state. Wisconsin experienced a similar development at the same time.

Workman (1958) has shown, in greenhouse experiments, that resistance to heptachlor has developed in onion maggots from two areas in Oregon.

In view of the development of the above conditions it is evident that further investigation on the effects of soil types on soil insecticides is necessary to understand why insecticides are extremely efficient in one soil type and almost worthless in another. Further, that with the apparent breakdown of insecticide recommendations in areas where maggot damage previously had been controlled, research embracing the study of insecticide resistance in onion maggots must be continued.

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Efficiency of Ethion in Oil Spray against California Red Scale and Citrus Red Mite[1]

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Abstract

Combinations of ethion with kerosene and medium-grade spray oil in aqueous mixture were tested in the laboratory. Ethion as the essential toxic agent of the kerosene mixture was very effective against eggs of nonresistant citrus red mites (*Panonychus citri* (McG.)). However, against eggs of citrus red mites resistant to organic phosphate insecticides there was a difference of more than a 15-fold increase in the LD_{95}. In combination with kerosene, malathion is more efficient than ethion against adult female California red scale (*Aonidiella aurantii* (Mask.)), but in combination with medium-grade spray oil efficiencies of additions of ethion or malathion were comparable.

Although the efficiency of a given deposit of spray oil is inversely proportional to the density of the scale population, this factor is nullified by incorporating ethion or malathion in oil at a deposit level in the upper range of efficiency against independent individual scale.

The trial deposits of ethion and malathion in spray oil were very effective in preventing settling of newly born scale. The selected trial dosage of medium-grade spray oil was as effective against eggs of citrus red mites resistant to organic phosphate insecticides as against those of a nonresistant population. A direct relationship between efficiency and increasing concentration of kerosene in aqueous mixture with a given concentration of ethion was found for adult female California red scale, but not for nonresistant citrus red mite eggs; however, a reduction in efficiency seemed to occur when the concentration of kerosene per 100 gallons of spray mixture was below 2 quarts for scale and 1 pint for mite eggs.

Results of trials with ethion against pests of deciduous fruits were reported by Dickinson (1958) and the acaricidal and insecticidal activity discussed.

In southern California, in general, a complex of pests must be considered in choosing a pest-control treatment for citrus. California red scale, *Aonidiella aurantii* (Mask.), and citrus red mite, *Panonychus citri* (McG.), are among the pests controlled by oil spray, and application timing is commonly arranged to coordinate control of both pests with a given application. Ebeling (1950, p. 461) pointed out the decline in efficiency of oil spray against California red scale from the foliage and fruit to the bark of branches and limbs. Although oil spray is a very effective treatment against citrus red mite, a single application annually is usually not sufficient on lemon trees and may not hold on orange trees in certain districts. One application in the fall and another in the spring (Newcomb 1947) is common treatment for lemon trees. Adverse reactions of orange trees to oil spray applied in the winter and spring are discussed by Ebeling (1950, pp. 461–2) and by Riehl *et al.* (1956).

Addition of a toxicant to oil spray to improve its pest control efficiency has been common practice. Evaluation of malathion for the control of California red scale has been reported by Carman (1956), and malathion alone or in combination with oil spray is now widely used in southern California. (Malathion does not contribute to the control of the citrus red mite.)

Resistance of the citrus red mite to organic phosphates was reported by Jeppson *et al.* (1958); a resistance factor of six times was found for ethion (Bio 1240) against the adult stage.

California red scales are viviparous. At birth the young scales have legs and are able to move about on the surface of the host plant for several hours while choosing a place to settle. The problems of spray coverage of a citrus tree are such that the efficiency of an insecticide against California red scale is enhanced by any residual activity of the deposit against scale crawlers or settlers.

In making mortality counts during laboratory studies of the efficiency of rotenone in oil spray against California red scale, Lindgren *et al.* (1945) used only scales separated from each other by at least the width of a scale, the reason for this procedure being "that the effectiveness of an oil spray has been found to be inversely proportional to population density. Scales draw oil from the surface of the fruit immediately adjacent to their bodies, and if they are situated too closely together, there is less oil available per scale for any given amount applied per unit area." Evidence presented by Riehl & LaDue (1952) demonstrates the direct relationship of mortality to increasing oil deposit.

Available information indicated that the insecticidal efficiency of ethion against California red scale would be obtained principally in combination with oil spray. The experiments reported here were conducted to ascertain for adult female California red scale and for citrus red mite eggs (1) the LD_{95} values of ethion as the primary toxic agent when applied in combination with a petroleum fraction and (2) the amount of ethion required in the mixture to increase the kill of oil spray from approximately 95 to 100%. Comparisons with malathion were used to estimate the performance of ethion in the field.

Materials and Methods.—Details of the experimental procedures followed in these studies are given in earlier papers by Riehl *et al.* (1952, 1953).

The nonresistant mites were obtained from an abandoned orange orchard near San Juan Capistrano which has not been treated for several years and which has never been treated with organic phosphate insecticides. The resistant mites were obtained from orchards in the Corona district having mite populations known to be highly resistant to organic phosphate insecticides (Jeppson *et al.* 1958).

For laboratory comparisons of the efficiencies of various

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The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

oils, it is usually preferable to restrict the mortality counts to scales at sufficient distances from one another so that each scale counted is an independent individual with respect to the available deposit of oil. However, this situation is not to be expected in the field, where infestations vary in density from isolated single individuals to clusters in which scale coverings are overlapping. It is therefore interesting to investigate the relationship of population density to the efficiency of combinations of ethion and oil spray against California red scale. To insure an adequate infestation in the laboratory procedures, it is necessary to transfer a slight excess of scale crawlers from the banana squash to the grapefruit. For trials against high population densities, the scales on grapefruits were left unthinned, while for trials in which each scale counted constituted an independent individual, the scales were thinned in the manner described by Riehl & LaDue (1952).

Under the rearing conditions in our laboratories, a new generation of young scales is born 38 to 40 days after the crawlers have been transferred to the grapefruit. The spray trials are applied at 36 days, and the mortality counts are made 21 days after the spray application. This interval is sufficient for scale crawlers to emerge and settle. The residual activity of the deposit against scale crawlers was measured by counting the live settlers per square centimeter at the time counts were made of the mortality of the adult female scale.

The 95% technical grade of ethion and malathion was used. To determine the LD_{95} attributable to ethion or malathion used in combination with oil spray, it in preferable to use a petroleum fraction which in itself does not contribute to the efficiency of the spray beyond that of its function as a vehicle for depositing and distributing the toxicant. Kerosene with a boiling range of 360° to 540° F. and a minimum unsulfonated residue of 92% was selected because it is a readily available petroleum fraction which by itself does not kill either California red scale or citrus red mite eggs. The kerosene was saturated with the dye Oil Red O used to determine the deposits colorimetrically (Riehl *et al.* 1953). A concentration of 10% ethion or malathion by volume was mixed with the dyed kerosene. The mixture of malathion and kerosene was not stable and had to be shaken vigorously each time a portion was taken to prepare a spray mixture. The kerosene containing the toxicant was applied in aqueous spray mixture; a series of concentrations were used to obtain a range of deposits.

In addition to the objective of calculated data for the LD_{95} and the dosage-mortality line attributable to the ethion used in combination with kerosene in aqueous spray mixture, the efficiency of ethion as a supplement to a full dosage of a conventional spray oil for citrus pest control was of interest. A California medium-grade (Erb 1932, Marshall 1932) emulsive spray oil was used at concentration of oil just sufficient for mortalities in the range of 90% of citrus red mite eggs or of the thinned adult female California red scale. The concentration of oil was held constant and the concentration of the toxicant, ethion or malathion, varied in a series of spray mixture combinations to determine the approximate minimum amount of toxicant required to increase the kill from the level of 90% to that of 100%.

If the insecticidal efficiency of ethion against California red scale occurs principally in combination with oil spray would the concentration of oil be a factor? In the trials conducted with 10% ethion in kerosene for the dosage-mortality line, the concentrations of both the ethion and the kerosene varied as the dosage was increased in the series of spray mixtures. To provide information on the function of the concentration of oil, the amount of ethion required for the respective LD_{95} values for citrus red mite eggs or California red scale was held constant and the concentration of kerosene varied in a series of spray mixture combinations.

DISCUSSION OF RESULTS.—The LD_{95} values of the solution of ethion or malathion in kerosene against eggs of citrus red mite collected from populations nonresistant or resistant to organic phosphate insecticides and against California red scale are given in table 1. While kerosene served to distribute the toxicant in relation to the test organism, the mortality was caused essentially by the toxicant. The data of table 1 show that ethion is highly efficient against eggs of the nonresistant citrus red mite; however, in comparison more than a 15-fold increase in deposit is required for the LD_{95} against eggs of the phosphate-resistant population. While it is not unexpected, it is notable that in these trials against the egg we found resistance to ethion similar to that observed by Jeppson *et al.* (1958) for the adult. The term "inefficient" is used for the results with malathion, since no appreciable mortality of citrus red mite eggs occurred with deposits of malathion comparable with those of ethion.

Comparison of the LD_{95} of ethion and malathion in kerosene against California red scale (table 1) indicates that *per se* malathion is more efficient than ethion against adult female California red scale. However, when the spray mixture was prepared with medium-grade spray oil, comparable increases in efficiency against California red scale occurred with additions of either ethion or malathion (table 2).

Comparison of the data of tables 1 and 2 for scale indicates that ethion is more effective in combination with medium-grade spray oil than with kerosene. The percentage kill of individual (hand-thinned) adult female California red scale (table 2) by a given dosage of spray oil

Table 1.—LD_{95} and probit analysis data of 10% ethion or malathion in kerosene for adult female California red scale and day-old eggs of citrus red mite.

Toxicant	LD_{95}, in Micrograms 10% Mixture per Sq. Cm.	Confidence Limits (% LD_{95})	Probit Analysis[a] $Y=a+b(X-\bar{x})$
Day-old eggs of nonresistant citrus red mites			
Ethion	14.9	6.4	$=3.97X+1.79299$
Malathion	Inefficient		
Day-old eggs of resistant[b] citrus red mites			
Ethion	256.7	20.6	$=1.79X+2.33518$
Malathion	Inefficient		
Adult female California red scales			
Ethion	159.9	4.2	$=3.75X-1.62494$
Malathion	102.7	30.9	$=1.67X+3.29322$

[a] Y = probit value for percentage mortality; X = $\log_{10}$ of deposit in micrograms per sq. cm. fruit surface.

[b] Resistant to organic phosphate insecticides.

Table 2.—Mean percentage mortality of day-old eggs of nonresistant and resistant citrus red mites and of adult female California red scales obtained with additions of various concentrations of ethion and malathion to a given concentration of California medium-grade spray oil.

	Ethion			Malathion		
Test Species	Ounces of Toxicant per 100 Gallons of Oil Spray Mixture	Per Cent Mortality	Live Settlers per Sq. Cm.	Ounces of Toxicant per 100 Gallons of Oil Spray Mixture	Per Cent Mortality	Live Settlers per Sq. Cm.
Mites						
Nonresistant	0	93.4	—	0	94.8	—
	0.38	99.2	—	0.38	—	—
	0.64	99.6	—	0.64	95.2	—
	1.28	100.0	—	1.28	96.2	—
	2.56	—	—	2.56	96.8	—
				5.12	98.6	—
Resistant[a]	0	90.3	—	0	—	—
	0.38	96.3	—	0.38	—	—
	0.64	96.5	—	0.64	—	—
	1.28	98.6	—	1.28	—	—
	2.56	99.2	—	2.56	—	—
				5.12	—	—
Scale						
Thinned	0	84.6	.9	0	83.0	1.6
	0.38	100.0	0	0.38	99.6	0
	0.64	100.0	0	0.64	99.8	0.2
	1.28	—	—	1.28	99.6	0
	2.56	—	—	2.56	99.6	0
				5.12	100.0	0
Unthinned	0	72.9	5.2	0	69.0	12.9
	0.38	99.5	0.2	0.38	—	—
	0.64	99.5	0	0.64	99.3	0
	1.28	99.2	0	1.28	99.5	0
	2.56	100.0	0	2.56	100.0	0
				5.12	100.0	0

[a] Resistant to organic phosphate insecticides.

(90 μg./sq. cm.) was increased from 84.6% to 100% by the addition of the equivalent of 0.38 ounce of ethion per 100 gallons of mixture. In comparison, the LD_{95} of scale of the solution of 10% ethion in kerosene (table 1) represents the equivalent of a concentration of approximately 13 ounces of ethion per 100 gallons, and the dosage of kerosene is about 10/7 that of the spray oil used in the trials reported in table 2. These results suggest a role spray oil might play in enhancing the contact of ethion with the scale. DeOng *et al.* (1927) demonstrated that kerosene and spray oil penetrate into the tracheae of California red scale and that the scale can expel the kerosense but not the spray oil. Ethion is completely soluble in kerosene and spray oil and could enter the tracheae in solution. The ethion would be held in the tracheae by the spray oil producing an increase in efficiency. On the other hand, a greater deposit of ethion in kerosene is apparently required to counteract the ability of the scale to expel the solution from the tracheae. In comparison, it appears that application in combination with spray oil is more critical to the efficiency of ethion than to that of malathion against California red scale.

In table 2 the concentration of medium-grade spray oil was selected to kill most but not all of the hand-thinned scales and was the same for the trials against the thinned and unthinned populations. As expected, the percentage kill of the oil alone (0 ounces of toxicant) was less for the unthinned scales than for the thinned scales. However, there was only a slight difference in the efficiency of combinations of ethion and malathion with spray oil against thinned versus unthinned scales. The unthinned scales were left, as they had settled on the grapefruit, and the population density varied from single scale to clusters of scales with the coverings overlapping. Every scale was examined for the mortality counts. Ethion in oil seems to be slightly more efficient against thinned scales than against unthinned scales and this would agree with the hypothesis of penetration into the tracheae.

Data for live scale settlers per square centimeter of fruit surface when the mortality counts of the adult females were made at 21 days after application are listed in table 2 for the various treatments. The results indicate the complete effectiveness of the deposits of ethion and malathion against the young scale which would have been able to settle and survive the effects of the given deposit of medium-grade spray oil. Efficacy against the young scale would contribute materially to the control efficiency of the treatment under field conditions.

The results shown in table 2 indicate that the dosage of oil selected was as efficient against eggs of citrus red mites resistant to organic phosphate insecticides as against those of a nonresistant population. In combination with

spray oil, the addition of 0.38 ounces of ethion increased the kill of eggs of nonresistant mites from 93.4 to 99.2%. In comparison, an addition of eight times as much ethion (2.56 ounces) was required to obtain 99.2% kill of eggs of resistant mites. As expected, the spray trials conducted for purposes of comparison show that combinations of ethion in oil spray are more efficient than those of malathion against citrus red mite eggs.

Comparisons of the data for resistant citrus red mites (tables 1 and 2) indicate that ethion is much more efficient against the eggs in combination with medium-grade spray oil than with kerosene. The LD_{95} of 256.7 (table 1) represents the equivalent of 20 ounces of ethion per 100 gallons of mixture, whereas in table 2, the percentage kill of the oil spray was increased from 90.3 to 96.3% by the addition of 0.38 ounces of ethion per 100 gallons. Although the concentration of kerosene (table 1) was dependent on the ethion requirement, the comparative actual volume was relatively high and represented eight times that of the concentration of spray oil used in the trials against the mite eggs (table 2). Against eggs of nonresistant citrus red mites, comparisons indicate that the concentration of ethion required in the mixture is about five times as much in combination with kerosene as with medium-grade spray oil. In contrast to California red scale, available information does not offer a ready hypothesis for the influence of petroleum molecular size on the efficiency of ethion against citrus red mite eggs.

Results of studies of the relationship of increasing concentrations of kerosene in the spray mixture to the efficiency of a given concentration of ethion against eggs of nonresistant citrus red mites and individual (hand-thinned) adult female California red scales are given in table 3. The data show a direct relationship between increasing concentrations of kerosene and efficiency against scale, but not against the mite eggs. A reduction in efficiency seemed to occur when the concentration of kerosene per 100 gallons of spray mixture was below 1 pint for mite eggs and 2 quarts for scale.

Table 3.—Relation of concentration of kerosene in the spray mixture to the efficiency of a given concentration of ethion against day-old eggs of nonresistant citrus red mites and against thinned adult female California red scales.

	Per Cent Mortality	
	Citrus Red Mite	California Red Scale
Concentration Kerosene per 100 Gal.	1.3 Oz. Ethion/ 100 Gal.	13.0 Oz. Ethion/ 100 Gal.
None	99.3	71.4
4 ounces	97.4	—
8 ounces	95.5	—
1 pint	100.0	71.2
1 quart	100.0	61.6
2 quarts	—	82.4
1 gallon	—	95.6

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Effects of Some Chlorinated Hydrocarbon Insecticides on Nematode Populations in Soils[1]

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ABSTRACT

DDT applied in the laboratory to a loam soil at the rate of 600 and 1,000 lbs./6″ acre had no effect on nematodes 24 days after treatment, whereas lindane at 200 lbs./6″ acre caused an 85% reduction of the total population, a 99% reduction of *Aphelenchus avenae* and *Acrobeloides* spp., and an 85% reduction of *Dorylaimus* spp. These differences were all significant. *Rhabditis* spp. or *Psilenchus* spp. were not affected.

Field investigations with heptachlor applied to a Carrington loam at 25 and 125 lbs./acre and aldrin at 25 lbs./acre showed that 5 weeks after treatment no significant effect on the nematode populations was obtained.

Miami silt loam plots were treated with aldrin, DDT, and lindane in 1954 and investigated in 1957 and 1958 to study the long-term effects of these insecticides on nematode populations. Aldrin applied at rates up to 200 lbs./6″ acre in 1954 (24.6 lbs./acre present in fall of 1957) had no effect. Significant differences in nematode populations were found between the untreated soils and the plots that had been treated with DDT at a rate of 1,000 lbs./6″ acre in 1954 (604 lbs./acre present in the fall of 1957) and lindane at 100 lbs./6″ acre in 1954 (33.5 lbs. in the fall of 1957). The lower rates of application had no effect.

A saprophagous species *Chiloplacus symmetricus* was always significantly more numerous on both the DDT-treated (1,000 lbs./acre) and lindane-treated (100 lbs./acre) plots. Nematodes of the order *Tylenchida* were not numerous on the lindane-treated plot.

From these investigations it seems that only some chlorinated hydrocarbon insecticides or their breakdown products influence nematode populations as a result of their direct or indirect effect on certain species or groups. Saprophagous species were often increased in number. Parasitic nematodes were often reduced slightly in numbers, but as few were found it was impossible to draw any conclusion.

Under field conditions, however, the nematode population was not seriously affected by the insecticides.

Although it has been established that accumulations of some of the chlorinated hydrocarbon group of insecticides or their breakdown products occur in the soil (Chisholm *et al.* 1951, Ginsburg 1955, Lichtenstein 1957) or persist over a relative long time (Lichtenstein & Schulz 1959, Lichtenstein & Polivka 1959), little is known of their effect upon the biological complex in the soil. Nematodes form a part of this complex, and in recent years we have become more aware of their importance. Some are obligate plant parasites, other are facultative parasites, and many are either saprophagous or predaceous. Some, therefore, perform a useful function and others reduce yields by their persistent attacks upon crops.

Insecticides have been used in an attempt to control specific nematodes, but the effect of these insecticides or their breakdown products upon the whole nematode group has received little attention. Grigor'eva (1952) found increased numbers of nematodes after treatment with 4 to 26 lbs./acre of BHC dust and Stockli (1952) also found increased numbers after treatment with DDT applied at the rate of 489 lbs./acre. The numbers were further increased 30 days later. BHC applied at 350 lbs./acre did not affect the nematode population. Satchell (1955) concludes no evidence has been found of wholesale or permanent destruction of soil fauna where DDT or BHC have been used at normal field rates of application. Clayton & Ellis (1947) found that BHC at 645 lbs./acre produced phytotoxic effects on plants but had no effect on incidence of rootknot nematodes. Adams (1955) was the first to record that BHC improved tree growth by reducing the numbers of *Xiphinema*, which may be the agent causing apple decline. It appeared, therefore, that insecticides might be effective against certain groups of nematodes.

The investigations reported herein were conducted to secure information on the short- and long-term effects of insecticide residues in agricultural soils on the different species and groups of nematodes.

METHODS AND MATERIALS.—To study the persistence and disappearance of chlorinated hydrocarbons, a Miami silt loam was treated in the spring of 1954 with aldrin at 2, 20, and 200 lbs./acre; lindane at 1, 10, and 100 lbs./acre; and DDT at 10, 100, and 1,000 lbs./acre.

The experimental field was divided into 12 plots, each 29 ft.×100 ft. with a 15-ft. buffer strip between. Nine of the plots were treated with the insecticides listed above, three were left untreated as check plots. Insecticides were sprayed in an emulsion form and rototilled in to a depth of 4 to 5 inches. After treatment, each plot was divided into two equal parts. One part was planted with various crops and the other was kept in fallow. For insecticide residue determinations, the experimental plots were analyzed periodically by specific chemical methods and bioassay procedures, to establish depletion curves (Lichtenstein & Schulz 1959).

For nematode investigations 40 cores per plot were taken from a grid pattern to a depth of 8 inches using a soil auger with a $\frac{3}{4}$-inch diameter. When samples were being taken to determine the vertical distribution of nematodes in the Miami silt loam plots, the sampling depth was 9 inches and each core was equally divided into three layers (1 to 3 inches, 4 to 6 inches, and 7 to 9 inches inclusive).

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The bulk samples were passed through a ¼-inch sieve and thoroughly mixed before being placed in cold storage until the nematodes were extracted.

Five hundred grams of field moist soil were used for nematode extraction from the Miami silt loam samples when cores to a depth of 8 inches had been taken. In all other cases the nematodes were extracted from 250 gms. field moist soil taken from the composite sample.

As soil moisture, organic matter content, and pH might influence nematode populations, determination of these factors was made using standard procedures.

The Cobb (1918) sifting and gravity method as modified by Christie & Perry (1951) was used in obtaining the nematodes: after thorough mixing the sample was placed in a 10″ enamel basin with the soil 3″ deep, covered with water and mixed until the soil particles were broken down. The muddy mixture was passed through a 10-mesh sieve into a second basin. The sediment left in the first basin was discarded. The whole process was repeated using 25-, 50-, 100-, and 200-mesh sieves (the 200-mesh sieve was used twice).

The stem of a funnel was closed with rubber tubing and a clip and filled with water. The residues on the sieves from 25 to 200 mesh were collected in a beaker that was covered with cotton sheeting, secured with a rubber band, and inverted into this funnel so that the water was above the opening of the inverted beaker. After 24 hours the nematodes had passed through the cotton and accumulated in the stem of the funnel. One hundred mls. of the water in the funnel were drawn off into a beaker and the nematodes present counted. This was done in a Syracuse watch glass which had been divided radially into 12 equal segments, each segment being subdivided to simplify counting under a stereoscopic microscope.

IDENTIFICATION.—Two lots of 50 nematodes each were taken from a collection by selecting them from random segments of the watch glass.

From these two samples the total number of nematodes present in each genus was calculated. The multiplication factor was obtained by dividing the total nematodes present in the sample by 50.

The short-term effects of DDT and lindane on nematode populations in a Miami silt loam were studied in the laboratory. Known amounts of insecticides in an acetone solution were pipetted onto the soil within a wide mouth glass jar. Thereafter, the soil was mixed by rolling horizontally in a tumbling machine for half an hour. The acetone was evaporated by passing a slight air stream over the soil. DDT was used at 500 p.p.m. (approximately 1,000 lbs./6″ acre) and 300 p.p.m. (approximately 600 lbs./6″ acre) and lindane at 100 p.p.m. (approximately 200 lbs./6″ acre). Checks were treated with solvent only.

To have plant growth in the experimental soils, peas were first germinated in quartz sand and then transplanted into the treated soils. The pots were watered to keep the soil moist.

Short-term effects of heavy applications of heptachlor and aldrin in a Carrington loam under field conditions were investigated. Heptachlor at 25 and 125 lbs./acre and aldrin at 25 lbs./acre were applied in an emulsion form to the experimental plots, the dilute emulsion being watered on in strips and then rototilled in to a depth of 4 to 5 inches. Solvent-emulsifier solutions, not containing any insecticide, were applied to the "check" plots. Additional untreated areas were used as controls.

RESULTS.—*Nematode Populations in Planted Areas, 3½ Years after Soil Treatments.*—Investigations were first carried out on all the planted areas of the Miami silt loam plots. The moisture content (about 17%), organic matter content (2.8%), and pH (7.2) were fairly uniform. Nematode counts made on those plots are shown in table 1. Nematode species present in the Miami silt loam are listed in table 2. Only in those plots treated with DDT at 1,000 lbs./acre (contained 604 lbs./acre at time of sampling) and lindane at 100 lbs./acre (contained 33.5 lbs./acre when sampled) were the populations such as to suggest any change might have been caused by the insecticides or their breakdown products.

The plot treated with lindane at 100 lbs./acre showed a two-third reduction of nematodes of the order Tylenchida. In the plot treated with DDT at 100 lbs./acre numbers of *Dorylaimus* sp. were low (5 compared with 68 in check). The saprophagous forms *Eucephalobus* sp. (184 compared with 60 in check) and *Chiloplacus symmetricus* (124 compared with 16 in check) were numerous.

Nematode Populations in Fallow Areas 3½ Years after Treatment with DDT and Lindane.—As the only differences in nematode populations in the planted areas were found in those plots that had received high application rates of DDT and lindane, further studies of those plots were conducted and laboratory experiments were made where DDT and lindane at high concentrations were used.

Samples were examined from the fallow areas of the DDT (1,000 lbs./acre) and lindane (100 lbs./acre) treated plots as well as from three check plots. The results are summarized in figure 1.

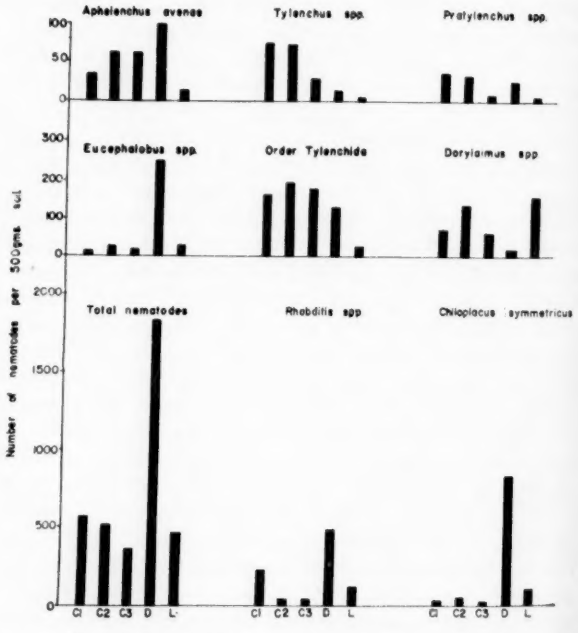

FIG. 1.—Nematodes found in the fallow areas of a Miami silt loam 3½ years after treatment with DDT and lindane. C_1, C_2, C_3 = untreated plots. Application rates: D = DDT 1,000 lbs./6″ acre. L = Lindane 100 lbs./6″ acre.

Table 1.—Nematodes found per 500 grams of soil of the planted areas of Miami silt loam plots 3½ years after treatment with DDT, lindane, and aldrin in the spring of 1954.

Nematodes	Plot[a]											
	CK1	CK2	CK3	A2	A20	A200	D10	D100	D1000	L1	L10	L100
Insecticide present fall of 1957 (lbs./6″ A.)				0	0.3	24.6	3.2	48.0	604.0	0	1.9	33.5
Rhabditis	168	91	35	210	39	30	14	22	9	164	64	49
Panagrolaimus								6		5		4
Eucephalobus	36	80	66	129	99	134	11	132	184	59	40	17
Acrobeloides	9	5	9	25	9	4		12	9	11		8
Acrobeles									5			
Chiloplacus	16	24	9	24	14	17	14	18	124	38	16	84
Tylenchus	32	30	31	16	18	21	21	12		32	12	7
Tylenchorhynchus											1	
Psilenchus			31				10					
Ditylenchus	5	15	6	15	23	17	7	12	9		4	
Helicotylenchus	21	35	4	88	5		60	37	10	6	53	24
Pratylenchus	39	76	62	101	39	34	39	66	59	16	76	4
Nothotylenchus			4		5	9					4	
Boleodorus	49	41	22	49	34	17	84	24		54	31	3
Pratylenchus		10										
Aphelenchus	50	15	65	97	66	30	17	36	6	44	6	12
Aphelenchoides	5	10				13		5				4
Plectus	5		8	9	5	17	7	8				
Mononchus					24		4	11			9	
Dorylaimus	80	50	75	25	97	76	74	162	5	91	87	152
Discolaimus			4					6				
Discolaimium				9								
Pungentus		10								6		
Tylencholaimus	17	6	9	16						5		
Xiphinema		5						30		7		
Dorylaimellus						5			4			
Diphtherophora						4						
Total	532	503	440	813	467	428	352	601	454	539	450	399

[a] CK 1, 2, 3 = Untreated plots.
A2, A20, A200 = Aldrin applied at 2 lbs., 20 lbs., 200 lbs. per 6″ acre.
D10, D100, D1000 = DDT applied at 10 lbs., 100 lbs., and 1,000 lbs., per 6″ acre.
L1, L10, L100 = Lindane applied at 1 lb., 10 lbs., 100 lbs. per 6″ acre.

The total nematode population shows a 3-fold increase on the DDT treated plot which is highly significant, as well as a 16-fold increase in the numbers of *Chiloplacus*. Also, *Chiloplacus* showed a significant increase on the lindane-treated plot, where at the same time significantly low numbers of nematodes in the order Tylenchida were found.

The numbers of *Dorylaimus* spp. were lower in the soil which had been treated with DDT at a rate of 1,000 lbs./acre in 1954. But this fact was not significant.

A fairly large increase in numbers of *Eucephalobus* in the DDT-treated plot (1,000 lbs./acre) was not significant because of the big variation found in the duplicate samples.

Other genera were present either in very small numbers or their numbers did not vary in treated soil compared with those found in the untreated soils.

Vertical Distribution of Nematodes in the Fallow Area, 4 Years after Treatment with DDT and Lindane.—As 84 to 96% of the insecticides present are concentrated in the top 3 inches (Lichtenstein 1958) investigations were

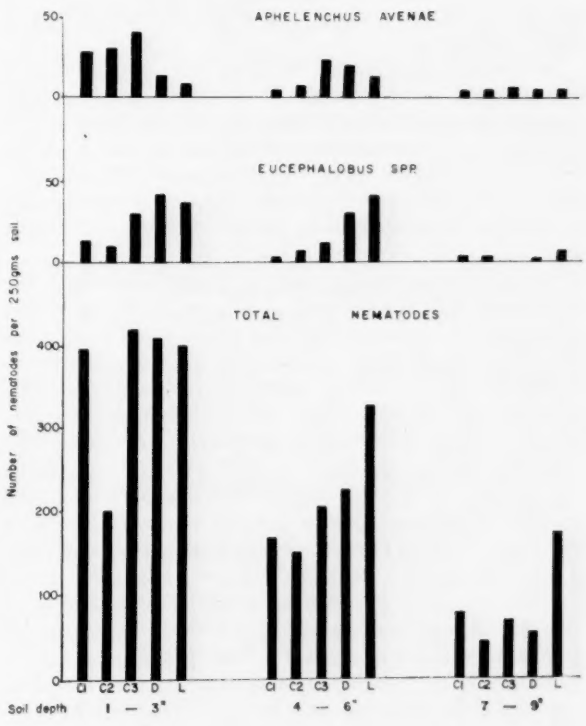

FIG. 2.—Vertical distribution of nematodes in fallow areas of a Miami silt loam 4 years after treatment with DDT and lindane. C_1, C_2, C_3 = untreated plots. Application rates: D = DDT 1,000 lbs./6″ acre. L = Lindane 100 lbs./6″ acre.

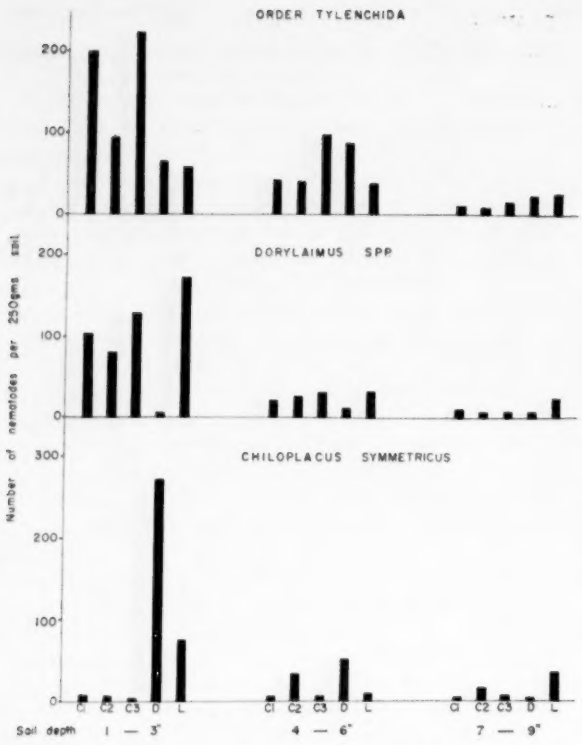
ORDER TYLENCHIDA
DORYLAIMUS SPP
CHILOPLACUS SYMMETRICUS
Number of nematodes per 250gms soil
C1 C2 C3 D L
Soil depth 1 — 3" 4 — 6" 7 — 9"

FIG. 3.—Vertical distribution of nematodes in fallow areas of a Miami silt loam 4 years after treatment with DDT and lindane. C_1, C_2, C_3 = untreated plots. Application rates: D = DDT 1,000 lbs./6″ acre. L = Lindane 100 lbs./6″ acre.

made of vertical distribution of nematodes in the soil. Most of the nematodes were found in the top 3 inches and only in this layer was any appreciable change present in populations.

In the plot treated with DDT at a rate of 1,000 lbs./acre 4 years previously, the number of *C. symmetricus* was again significantly increased and the number of *Dorylaimus* sp. significantly decreased. In the lindane-treated plot (100 lbs./acre) the numbers of *C. symmetricus* were also significantly increased. Though the nematodes in the order Tylenchida were low in numbers on this plot, this fact was not significant (figs. 2, 3).

In the *laboratory experiments* immediately after soil treatment with acetone only, an average of 180 nematodes/250 gm. soil were found in the check. Twenty-four days later (fig. 4) 550 nematodes/250 gm. soil were found. Nematode populations in soil treated with DDT at 600 and 1,000 lbs./6″ acre did not differ from checks. Lindane at 200 lbs./6″ acre had some phytotoxic effects, but caused a highly significant reduction in total numbers. An average of 68 nematodes was found/250-gm. soil sample. *Aphelenchus avenae* was the dominant species in the check and DDT-treated soil, but had almost completely disappeared from the lindane-treated soils (1/250 gm. soil compared with 259 in checks). The population of *Acrobeloides* spp. was significantly reduced by lindane (1 compared with 24 in checks) as was *Dorylaimus* spp. (7 in lindane treated and 65 in checks). The genera *Rhabditis* spp. and *Psilenchus* spp. were not notably affected.

Nematode Populations in a Carrington Loam after Treatment with Heptachlor and Aldrin.—Heptachlor has been recorded in the literature as having nematocidal properties (Tarjan 1953). Therefore, it was felt that sampling different soil layers soon after application might show its effectiveness, since most of the insecticide would still be concentrated in the top 3″ soil layer.

Samples taken from one replicate 15 days after treatment with heptachlor at a rate of 125 lbs./acre showed only half the nematodes present in each layer (1″ to 4″, 5″ to 8″, 9″ to 12″) examined as compared with the check plot treated with solvent only. However, no significant change was found when all the plots (heptachlor, solvent only, and untreated) were sampled 5 weeks after treatment.

Nematodes were also apparently not affected 5 weeks after treatment with aldrin at 25 lbs./acre.

CONCLUSIONS.—One important fact ascertained by this investigation is the great similarity in the nematode populations found within the differently treated plots. As relatively large amounts of DDT, lindane, and aldrin had been in the soil over periods of 3 to 4 years, primary or secondary effects on nematodes might have been expected.

Lack of replication in different soil types has made any conclusions only tentative, but it might be asserted that no serious upset of the nematode population has occurred.

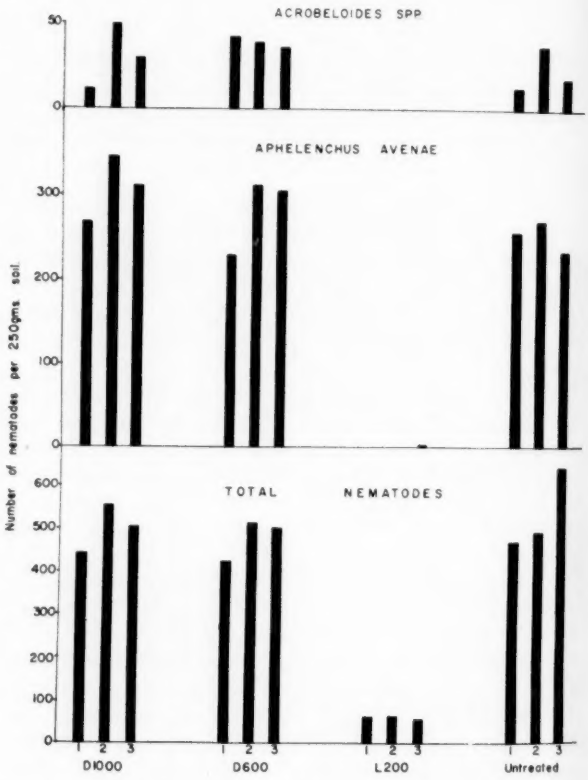

FIG. 4.—Nematodes found in a Miami silt loam 24 days after treatment with DDT and lindane (laboratory experiment). Application rates: D1000 = DDT 1,000 lbs./6″ acre. D600 = DDT 600 lbs./6″ acre. L200 = Lindane 200 lbs./6″ acre 1, 2, 3 = Replicates.

Table 2.—A list of nematode species present in Miami silt loam plots.[a]

Acrobeles ctenocephalus Thorne, 1925
Acrobeloides bütschli (deMan, 1884) Steiner & Buhrer, 1933
Acrobeloides minor (Thorne, 1925) Thorne, 1937
Acrobeloides tricornis (Thorne, 1925) Thorne, 1937
Aphelenchoides spp.
Aphelenchus avenae Bastian, 1865
Boleodorus thylactus Thorne, 1941
Chiloplacus symmetricus (Thorne, 1925) Thorne, 1937
Ditylenchus spp.
Eucephalobus oxyuroides (deMan, 1876) Steiner, 1936
Eucephalobus striatus (Bastian, 1865) Thorne, 1937
Dorylaimus bastiani Bütschli, 1873
Dorylaimus carteri Bastian, 1865
Dorylaimus clavicaudatus Thorne & Swanger, 1936
Dorylaimus monhystera deMan, 1880
Dorylaimus obscurus Thorne & Swanger, 1936
Dorylaimus pratensis deMan, 1880
Dorylaimus sublabiatus Thorne & Swanger, 1936
Dorylaimus spp.
Helicotylenchus spp.
Mononchus parabrachyurus Thorne, 1924
Panagrolaimus subelongatus (Cobb, 1914) Thorne, 1937
Plectus spp.
Pratylenchus minyus Sher & Allen, 1953
Pratylenchus brachyurus (Godfrey, 1929) Filipjev, 1934
Pratylenchus spp.
Psilenchus aberrans Thorne, 1949
Psilenchus hilarulus deMan, 1921
Psilenchus gracilis Thorne, 1949
Rhabditis spp.
Tylencholaimellus striatus Thorne, 1939
Tylenchorhynchus maximus Allen, 1955
Tylenchus costatus deMan, 1921
Tylenchus davainii Bastian, 1865
Tylenchus exiguus deMan, 1876
Xiphinema americanum Cobb, 1913
Xiphinema diversicaudatum (Micoletzky, 1927) Thorne, 1939

[a] Unidentified species usually were represented only by immature forms.

The most outstanding change has been in the Miami silt loam plot treated with DDT at 1,000 lbs./acre in 1954 and containing at the time of sampling (fall of 1957) 604 lbs./6″ acre, in which the numbers of *Chiloplacus symmetricus* both in spring and in the fall were significantly increased. *Eucephalobus* (another saprophagous genus) has shown smaller increases. The greatest increase occurred in the top 3 inches of soil where 80% of the insecticide recovered from a 9-inch sample, was concentrated.

DDT at 600 or 1,000 lbs./acre had no effect on the population of nematodes in plots containing Miami silt loam in 24 days, whereas lindane at 200 lbs./acre caused a significant reduction of certain species. Whether this condition occurred after initial application to the field plots is not known, but the population in these plots $3\frac{1}{2}$ years after treatment varied only slightly from the untreated plots.

Observations on Carrington loam plots treated with heptachlor at 25 and 125 lbs./6″ acre 5 weeks after treatment showed no difference in nematode populations between the treated plots, the untreated plots, and those receiving solvent only. Heptachlor had no short-term effect. Its long-term effect is not known. Thus it is extremely unlikely that the nematode population will be affected by prolonged or concentrated use of DDT or aldrin applied either to the soils or crops. The saprophagous species may be increased but the parasitic species probably remain unaffected.

Lindane at the normal field dosages has probably no effect, but very high rates of application which produce phytotoxic effects on plants might control certain nematodes. When a high initial dosage had reduced naturally over a period of years no outstanding effects upon the nematode populations were observed.

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Effectiveness of Aerosol Formulations Containing Methoxychlor and Other Insecticide-Repellents against Biting Flies on Cattle, and Analyses of Milk from Treated Animals[1]

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ABSTRACT

Aerosol containers charged with 1, 2, and 4% of methoxychlor in various combinations with N,N-diethyl-*m*-toluamide, Thanite® (isobornyl thiocyanoacetate—82%), MGK R-326 (di-*n*-propyl isocinchomeronate), pyrethrins, and other ingredients were released on lactating cows by means of an automatic photoelectric device. The major components of the device consisted of a rectangular-shaped wooden frame on which were mounted a photoelectric relay unit and a triggering mechanism. Three aerosol containers were strategically located on the frame. The aerosol spray was released instantly as cattle entered the frame and stopped as soon as they left. On the average, actual spraying time per animal was 1.5 seconds; 150 animals were treated by a set of three 12-ounce containers, a dosage of approximately 7 ml. being discharged on each animal. The spray was applied once daily. Milk from both treated and untreated cows was collected and analyzed once or twice a week.

Results of the tests made over a period of 14 weeks showed that the aerosol formulations gave consistently excellent control of the horn fly, *Siphona irritans* (L.), but limited protection against the stable fly, *Stomoxys calcitrans* (L.). None of the milk samples analyzed showed a detectable amount of methoxychlor.

The current price for the parts of the automatic device was between $20 and $25, and the base cost of the containers and aerosol ingredients averaged between 0.6 cent and 1 cent per animal per application.

Tests of treatments containing methoxychlor against biting flies on cattle conducted by the authors during 1957 showed that methoxychlor residues were detected in the milk of treated animals when such treatments were applied with an electric-eye-controlled sprayer. The authors also reported that an oil spray, containing methoxychlor in combination with Lethane 384®, (2-(2-butoxyethoxy) ethyl thiocyanate) synergized pyrethrins and butoxy polypropylene glycol provided excellent control of the horn fly, *Siphona irritans* (L.), and stable fly, *Stomoxys calcitrans* (L.), (Cheng *et al.* 1958).

During the summer of 1958 further tests on biting fly control were conducted. Aerosol formulations containing methoxychlor in various combinations with diethyltoluamide (N,N-diethyl-*m*-toluamide), Thanite® (isobornyl thiocyanoacetate—82%), MGK R-326 (di-*n*-propyl isocinchomeronate), pyrethrins, and other ingredients were released on cattle by means of an automatic photoelectric device, and milk from both treated and untreated cows was collected and analyzed periodically. The purpose of the investigation reported in this paper was to study the effectiveness of the aerosol formulations against horn flies and stable flies, and to determine whether aerosol sprays containing methoxychlor could be used on lactating cows without contaminating milk from the treated animals.

EXPERIMENTAL PROCEDURE.—The automatic photoelectric device was adapted from the electric-eye-controlled sprayer (Cheng *et al.* 1957). The major components of the device consisted of a rectangular-shaped wooden frame on which were mounted a photoelectric relay unit and a triggering mechanism. Three aerosol containers were attached to the frame; two were placed vertically 17 inches from the ground, one on each side of the frame, and a third one was installed under the cross bar at the center of the top. A 6-volt battery or 110 volts A.C. supplied the electric power (fig. 1). The aerosol spray was released instantly as cattle entered the wooden frame, and stopped as soon as they left. On the average, actual spraying time per animal was 1.5 seconds; 150 animals were treated by a set of three 12-ounce containers, a dosage of approximately 7 ml. being discharged on each animal. The device was installed on the University Farm No. 12 of The Pennsylvania State University.[3]

Thirty-eight lactating Holsteins were divided into treated and untreated groups of 28 and 10, respectively. The two groups occupied different sections of the stable. The untreated group was led to one pasture before spraying started. The spraying device was located about 30 yards from the stable and the cows to be treated passed through it and were sprayed early in the morning, after being milked, while on their way to another pasture. Any physical contact between members of the two groups was thus avoided.

The aerosol containers used in the tests were charged with the following formulations: A. 1% methoxychlor, 15% diethyltoluamide, 24% deo-base, and 60% propellent; B. 1% methoxychlor, 3% Thanite, 36% Deobase, and 60% propellent; C. 2% methoxychlor, 2% MGK R-326 (di-*n*-propyl isocinchomeronate), 2% MGK 264 (N-octyl bicycloheptene dicarboximide), 34% petroleum distillate, and 60% propellent; D. 2% methoxychlor, 0.2% pyrethrins, 2% piperonyl butoxide, 45.8% petroleum distillate, and 50% propellent; E. 4% methoxychlor, 0.2% pyrethrins, 2% piperonyl butoxide, 43.8% petroleum distillate, and 50% propellent.[4]

During the experimental period of 14 weeks, spraying was suspended on several occasions when weather was

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[4] Aerosol containers and contents were generously donated by Hercules Powder Co., Wilmington, Delaware; McLaughlin Gormley King Co., Minneapolis, Minnesota; and Fairfield Chemical Division, Baltimore, Maryland.

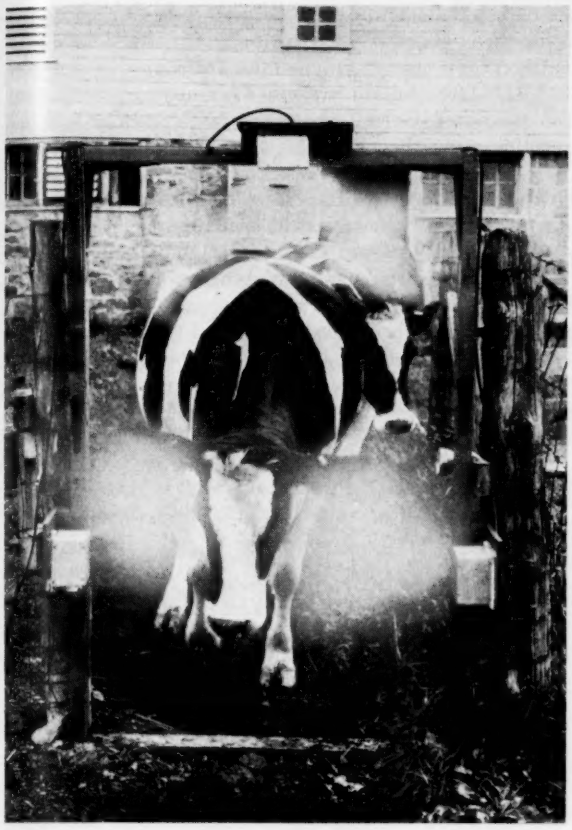

FIG. 1.—Dairy cows self-treating with an aerosol spray released by the automatic photoelectric device installed near a stable.

unfavorable or the spray material was changed. In the latter case, the animals were washed thoroughly with soap and water, and several days were allowed to pass before a new formulation was used. A total of 20 applications was made with formulation A, 13 with B, 6 with C, 16 with D, and 14 with E.

Fly counts made during pretreatment periods showed no significant difference in the degree of infestation between the treated and untreated groups. After treatments began, the flies were counted twice a day, at 6 and 11 hours after spraying. The mean number of flies per animal was determined by counting the flies on 10 animals selected at random in each group.

To determine methoxychlor residues in milk, 1-quart samples of milk were collected for chemical analysis from seven of the animals in each group. The collections and analyses were made once or twice a week as shown in table 2. These samples were extracted by the method of Carter (1947) and the methoxychlor determined by the method of Claborn & Beckman (1952) as described in a previous paper (Cheng *et al.* 1958). Long experience with this method has demonstrated that it has a sensitivity of 0.01 p.p.m. of methoxychlor.

RESULTS AND DISCUSSION.—Results of treatments with aerosol sprays are shown in table 1. The per cent reduction of horn flies and stable flies shown in the table is an average of the results for the 14-week period. Formulation A, containing 1% methoxychlor and 15% diethyltoluamide, achieved 96.7% reduction of horn flies and 48.3% reduction of stable flies. Formulation B, consisting of 1% methoxychlor and 3% Thanite, caused a reduction of 93.4% of horn flies and 30.7% of stable flies. Formulation C, containing 2% each of methoxychlor, MGK R-326, and MGK 264, reduced horn flies by 95.3% and stable flies by 41.9%. Formulation D, containing 2% methoxychlor, 0.2% pyrethrins, and 2% piperonyl butoxide, caused a reduction of 98.6% of horn flies and 48.1% of stable flies. Formulation E was the same as formulation D except that it contained 4% methoxychlor instead of 2%. It achieved 98.9% reduction of horn flies and 61.5% reduction of stable flies.

Approximately 50% of the stable flies counted on the treated animals were found on their heads, which were not sprayed during the early part of the tests. In the latter experiments, the heads were treated but part of the spray material was washed away by drinking water and dew.

Analyses of the milk samples showed that within the sensitivity of the method employed (0.01 p.p.m.), no detectable amount of methoxychlor was present (table 2).

The results stated above indicated that all the formulations tested provided excellent control of horn flies but none gave effective protection against stable flies. It is possible that the concentration of some of the principal ingredients in the formulations was too low to be effective against stable flies. This was substantiated by the results obtained with formulations D and E; when the concentra-

Table 1.—**The effectiveness of aerosol sprays containing methoxychlor released by an automatic photoelectric device[a] on dairy cattle for biting fly control. Centre County, Pennsylvania. June to September, 1958.**

FORMULATIONS[b]	PERIODS OF TREATMENT	NO. DAILY APPLICATIONS	MEAN NO. HORN FLIES PER COW PER COUNT[c]		PER CENT REDUCTION	MEAN NO. STABLE FLIES PER COW PER COUNT[c]		PER CENT REDUCTION
			Treated[d]	Untreated[d]		Treated[d]	Untreated[d]	
A	June 24–July 18	20	2.7	82.6	96.7	18.6	36.0	48.3
B	July 23–Aug. 8	13	4.4	66.3	93.4	36.6	52.8	30.7
C	Aug. 12–21	6	1.9	39.6	95.3	35.5	61.1	41.9
D	Aug. 24–Sept. 12	16	0.7	50.3	98.6	24.3	46.8	48.1
E	Sept. 13–27	14	0.3	26.8	98.9	11.5	29.9	61.5

[a] At the rate of 7 ml. per cow.
[b] See text for description of ingredients.
[c] Flies were counted at both 6 and 11 hours after spraying.
[d] 28 and 10 lactating Holsteins in the treated and untreated group respectively.

Table 2.—Residues of methoxychlor in milk collected from cows[a] treated with aerosol sprays containing methoxychlor by means of an automatic photoelectric device. Centre County, Pennsylvania. June to September, 1958.

Formulations (See Text)	Dates of Application	Milk Collected and Analyzed on	Methoxychlor Residues in Milk
A	June 24–28; 30 July 1–5; 7–12; 16–18	July 10; 14; 17; 21	Not detectable
B	July 23–26; 28–29; 31 Aug. 2; 4–8	July 24; 28; 31 Aug. 8	Not detectable
C	Aug. 12–15; 19; 21	Aug. 15; 22	Not detectable
D	Aug. 24–Sept. 3 Sept. 5–6; 8–9; 12	Aug. 29 Sept. 4; 12	Not detectable
E	Sept. 13–20; 22–27	Sept. 19; 23; 26; 30	Not detectable

[a] Seven lactating Holsteins.

tion of methoxychlor was increased from 2% in D to 4% in E, the effectiveness of the spray against stable flies was appreciably enhanced. The unsatisfactory performance of the formulations against stable flies may be partially attributed to the habit that cattle have of wading through mud holes or streams, which inevitably washes away the spray material on the legs. Furthermore, the extraordinary power of perception and selection possessed by stable flies was demonstrated by the fact that an average of 48.4% of the stable flies counted on the treated animals were located on parts of their heads and faces, to which the spray was not applied or from which it had been washed away in the course of drinking water or grazing wet grass. The development of water-repellent spray materials might help to overcome such difficulties. Finally, it has been pointed out that no detectable amount of methoxychlor was found in milk from animals treated with the aerosol formulations. This method of application appears to permit the use of methoxychlor without contaminating the milk, and thus should comply with the requirements of the Food and Drug Administration. Further experiments with aerosol formulations containing other promising toxicants, which might be more effective against stable flies, are planned for the coming season.

The investigation reported above indicated that the aerosol treatment of cattle was economical and labor saving. The current price for the parts of the automatic dedevice was between $20 and $25, and the base cost of the containers and aerosol ingredients averaged between 0.6 cent and 1 cent per animal per application.

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Exploratory Studies on Gamma Radiation for the Sterilization and Control of *Anopheles quadrimaculatus*[1]

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Abstract

In laboratory studies on the effect of gamma radiation on *Anopheles quadrimaculatus* Say, it was found that dosages of 8,865 to 12,900 r applied in the pupal or adult stage were required to cause complete sterility. Irradiated females mated to unirradiated males produced no eggs, whereas unirradiated females mated to irradiated males produced a normal number of eggs but none hatched. When irradiated males were introduced into caged populations of normal males and females at ratios of 4:1:1 or less, usually no reduction in the total number of viable eggs was produced, but at ratios of 6:1:1 and 10:1:1 there was a reduction of about 80%. The dosages (r) required to cause 50% and 100% mortality in 48 hours were: for eggs, 2,600 and >11,000; for larvae, 32,000 and 120,000; for pupae, 22,000 and 40,000.

The eradication of the screw-worm, *Callitroga hominivorax* (Cqrl.), from the island of Curaçao by the release of sterilized flies (Bushland *et al.* 1955, Baumhover *et al.* 1955) led to consideration of other pests that might be susceptible to eradication by this method. The method appeared to hold some promise for use against certain anophelines. These mosquitoes do not pass the winter in the egg stage, and consequently spring populations are usually low. Even if not suitable for use alone, the method might be useful in combination with others. Considerable effort is devoted to antimalaria campaigns, and sterilized insects might be released to apply the coup de grâce to a population already depleted by other control measures, the survivors of which might not be reached by any other means. Laboratory rearing procedures are available and it seems probable that mass rearing and irradiation techniques could be developed. Preliminary laboratory studies to evaluate the method against a representative anopheline appeared to be justified.

Laboratory studies were conducted to determine the effect of gamma radiation on *Anopheles quadrimaculatus* Say in various stages of development, and the results of adding sterilized males to caged normal populations. Two cobalt-60 radiation sources were used. One gave an aver-

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age dose rate of approximately 400 r and the other 1,000 r per minute over the useful volume. The use of these sources in determining the effects of gamma radiation on some other insects affecting man has been described by Cole *et al.* (1959).

IRRADIATION OF 1-DAY-OLD PUPAE.—Tests were made to determine the effect of irradiation in the pupal stage on the fertility of adults. Day-old pupae were confined in groups of 50 in small polyethylene bottles and exposed to dosages of 3,200, 6,400, and 12,900 roentgens in the smaller radiation source. After exposure each group was transferred to a screen holding cage, where adult emergence occurred. Fifty untreated pupae were utilized as a check. The adults were offered a human blood meal, honey, and a fresh supply of boiled water each day until all had died. Daily records were kept on adult emergence and mortality and the number and fertility of eggs laid. The average results in two duplicate series of tests are given in table 1.

The irradiation of the pupae did not reduce or delay adult emergence but it apparently did weaken some of the mosquitoes, since the mortality during the first 3 days was considerably higher among the irradiated insects than among the checks. However, the irradiated mosquitoes that survived the first 3 days lived about as long as the checks.

All irradiated groups laid some fertile eggs. Mosquitoes of the first series exposed to 12,900 r produced 80 fertile eggs on a single day and two infertile eggs the following day, whereas those of the second series laid no eggs. More fertile eggs were obtained from the mosquitoes exposed to 3,200 r than from the checks. It is doubtful, however, that the increases in egg production are significant.

Table 1.—Effect of irradiation of day-old pupae on the emergence, life span, and fertility of the ensuing adults of *Anopheles quadrimaculatus*.

Exposure (Roentgens)	Time After Exposure (Days)	Per Cent Emergence	Per Cent Mortality		Total Number of Fertile Eggs Laid
			Males	Females	
12,900	1	75	41	16	0
	2	97	49	27	0
	3	—	54	27	0
	7	—	76	53	0
	14	—	99	96	80
	21	—	100	100	0
6,400	1	67	19	13	0
	2	92	28	24	0
	3	—	28	28	0
	7	—	84	39	238
	14	—	97	70	842
	21	—	100	100	160
3,200	1	75	31	14	0
	2	94	44	17	0
	3	—	44	30	0
	7	—	73	42	621
	14	—	100	78	1,959
	21	—	100	100	640
Check	1	81	0	0	0
	2	91	20	6	0
	3	—	20	6	0
	7	—	74	30	488
	14	—	100	77	1,539
	21	—	100	100	488

Table 2.—Effect of 8,865 r of gamma irradiation on the survival and egg production of *Anopheles quadrimaculatus* exposed as pupae.

Age of Pupae When Exposed (Hours)	Survival (4 Tests)		Egg Production (2 Tests)	
	Per Cent Adult Emergence	Per Cent Survival After 3 Days	Total No. of Eggs Laid	Per Cent Hatch
1	74	4	0	0
4	91	31	0	0
8	93	55	657	0.75
12	92	63	150	0
16	87	40	43	2
24	96	53	128	0
Check	93	57	3,002	92

IRRADIATION OF PUPAE OF VARIOUS AGES.—Tests were run to determine the effect of exposure to 8,865 r in the smaller source on pupae of various ages. In each age group 50 pupae were exposed and 50 unexposed pupae were used as a check. The results are given in table 2. Three days after emergence adult mortality was highest among the groups exposed as 1- and 4-hour-old pupae. Many of these adults had been unable to disentangle themselves from their pupal cases or were drowned. Eggs were obtained from groups exposed as 8- to 24-hour-old pupae, but not more than 2% hatched.

IRRADIATION OF YOUNG ADULTS.—Adults exposed to 1,500 or 2,500 r of gamma ray radiation in the smaller source within 24 hours after emergence laid a normal number of eggs, all of which hatched. At 5,000 r the number and fertility of the eggs was slightly reduced. At 8,865 r only a few eggs were laid, none of which hatched (table 3). The numbers of eggs deposited by mosquitoes receiving 1,500 and 2,500 r were almost twice the number deposited by the checks. Further tests will be needed to determine if the increases in egg production are due to the irradiation.

EFFECT OF ADDING IRRADIATED MALES TO A POPULATION OF NORMAL MALES AND FEMALES.—To determine the effect of releasing various proportions of sterilized males in normal populations of caged adults, day-old pupae confined in small polyethylene bottles were exposed to 11,820 r in the larger radiation source and then transferred to pint jars of water in groups of 50 to 100. A 10-ounce conical waxed paper cup with 1 inch of the tip re-

Table 3.—Effect of gamma ray irradiation within the first 24 hours[a] of adult life on the fertility of *Anopheles quadrimaculatus*.

Dosage (Roentgens)	Number of Eggs Laid	Per Cent Hatched
1,500	6,143	100
2,500	6,852	100
5,000	2,200	89
8,865	174	0
None	3,470	100

[a] 60 mosquitoes.

Table 4.—Egg production and fertility in caged populations of *Anopheles quadrimaculatus* composed of various proportions of irradiated (R) and normal (N) males and females.

Ratio (R♂:N♂:N♀)	Population Components				Number of Tests	Average Number of Eggs per Female		Per Cent of Eggs Hatched
	R♂	R♀	N♂	N♀		Laid	Hatched	
1:1:1	25	0	25	25	3	99	74	74
2:1:1	50	0	25	25	4	66	56	85
3:1:1	75	0	25	25	2	54	23	42
4:1:1	100	0	25	25	3	123	65	53
6:1:1	150	0	25	25	1	20	8	42
10:1:1	250	0	25	25	4	30	8	28
—	0	25	25	0	2	0	0	—
—	25	0	0	25	2	47	0	0
—	0	0	25	25	9	47	45	96

Table 5.—Dosages (r) of gamma irradiation required to cause mortality of eggs, larvae, and pupae of *Anopheles quadrimaculatus*.

Lethal Dosage	Eggs	Larvae	Pupae
24-hour mortality			
LD-50	—	125,000	48,000
LD-90	—	170,000	100,000
Min. D-100	—	180,000	—
48-hour mortality			
LD-50	2,600	32,000	22,000
LD-90	11,000	80,000	39,000
Min. D-100	—	120,000	40,000

moved was placed over the mouth of each jar to funnel the emerging adults into a cylindrical screen cage. Mosquitoes to be used in mating experiments were separated according to sex when less than 20 hours old to assure that they had not mated. The sexes were determined by anesthetizing young adults with carbon dioxide and examining each specimen individually. As a check on the adequacy of the isolation procedure, enough of the females were separated so that a control group of supposedly virgin females could be maintained, and this group laid no eggs. The experimental populations were established in rearing cages within 20 hours after the adults emerged.

The results of this experiment are given in table 4. Irradiated females mated to unirradiated males produced no eggs, whereas unirradiated females mated to irradiated males produced a normal number of eggs, but none hatched. When irradiated males were introduced into populations of normal males and females at ratios of 4:1:1 or less, egg production was at or above the normal level, with the result that, although the fertility was slightly reduced, there was usually no reduction in the total number of viable eggs. At ratios of 6:1:1 and 10:1:1, however, both the number and fertility of eggs were reduced, with a total reduction in viable eggs of about 80%.

Multiple Mating.—In laboratory tests to obtain information on multiple matings, males and females were confined together for various periods and oviposition records were maintained for 3 weeks. The total number of eggs laid by various groups of females was as follows:

(1) 25 males, 25 females, together 21 days	1,353
(2) 25 males, 25 females, together 3 days	1,757
(3) 10 males, 25 females, together 21 days	1,105
(4) 10 males, 25 females, together 3 days	657
(5) No males, 25 females	0
(6) 25 males from (2) above, 25 virgin females, together from the 3rd to 15th day	932

More than one mating by the males was indicated. Twenty-five females confined for 21 days with 10 males laid almost as many eggs as those confined with 25 males, and when males were transferred to a second group of virgin females after 3 days, 932 eggs were obtained. Multiple matings did not occur in quick succession, however, as 3 days was not sufficient to permit 10 males to cause maximum fertilization of 25 females. Since the females confined with 25 males for only 3 days laid the most eggs, frequent matings by females appear to be unnecessary.

Lethal Doses of Irradiation.—Eggs, fourth-instar larvae, and pupae were exposed to five to eight dosages of irradiation in the larger source to determine the dosages required to cause mortality in 24 and 48 hours. One hundred individuals of each stage were exposed to each dosage. All exposures were made in duplicate, The results, summarized in table 5, show that the eggs were susceptible to the lowest dosages and larvae required the highest. Data for 24-hour mortality of eggs could not be obtained, since only 51% hatch had occurred at this time.

Conclusions.—The demonstration that *A. quadrimaculatus* mosquitoes can be sterilized by gamma irradiation in the pupal or adult stage, and that the release of adequate numbers of sterile males in a normal population results in a substantial reduction in the number of viable eggs produced, indicates sufficient promise to justify further exploration of the possibilities of the use of sterilized anophelines as a control method. The practicability of the method would depend on the species of mosquito, the development of the most efficient procedures for inducing maximum sterilizing effects, the size of the area to be treated, the abundance of the normal mosquitoes, the importance of obtaining complete eradication, which is difficult to obtain by other means, and the financial resources available. Although at first glance this appears to be an expensive method, it might be more economical in certain circumstances than the current methods.

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European Red Mite Control and Population Studies on Apple in Wisconsin[1]

EARL R. OATMAN[2]

ABSTRACT

Genite® (2,4-dichlorophenyl benzenesulfonate), Mitox® (*p*-chlorobenzyl *p*-chlorophenyl sulfide), and ovex applied as pre-blosson acaricides provided season-long control of the European red mite, *Panonychus ulmi* (Koch), on apples in Door County, Wisconsin, during 1956 and 1957. Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), Chlorobenzilate® (ethyl 4,4′-dichlorobenzilate), demeton, Kelthane® (1,1-bis(*p*-chlorophenyl)-2-2-2-trichloroethanol), and Mitox, used as summer foliar sprays, were effective in reducing and maintaining the mite populations at low levels for the remainder of the season. The results of 3 years' study of the European red mite populations are given. There were five generations in 1956 and 1957 and four generations in 1958. The populations normally reached their highest peak at the end of July and then abruptly declined. The first summer eggs were oviposited by blossom time. Overwintering eggs were laid the first week in August. The highest number of eggs were oviposited by the first summer generation adults. European red mite populations on treated trees exceeded those on the check (untreated) trees the first of September, remained at a higher level until the end of the season, and oviposited more overwintering eggs than populations on the check trees. The number of overwintering eggs oviposited in the treated plots were proportional to the degree of control obtained. The 1958 populations, which developed from overwintering eggs laid on the trees in the 1957 treated plots, were higher and reached peaks earlier than those on the trees which had been a part of the check plot in 1957. Predaceous typhlodromid mites, the only predators observed throughout the studies, were in very low numbers in the 1956 and 1957 check plots. Typhlodromids were more numerous on the unsprayed trees in 1958. They were present the first week in July; gradually increased in numbers; and finally surpassed the European red mite population in mid-August. Two species were present. The primary one, *Typhlodromus fallacis* (Garman), comprised 95% of the population.

The European red mite, *Panonychus ulmi* (Koch), is endemic in apple orchards of Door County, Wisconsin. Under optimum (warm, dry) weather conditions the species is capable of attaining high populations whose feeding may cause severe bronzing of the foliage. As a result, the vitality of the tree, the yield, and the quality and flavor of the fruit may be adversely affected (Lathrop 1951; Chapman *et al.* 1952, Lienk *et al.* 1956).

Fortunately for Door County apple growers the growing seasons are usually characterized by mild temperatures and sufficient rainfall (fig. 1). Under these conditions the European red mite populations fluctuate at or below destructive levels. Also, the two-spotted spider mite, *Tetranychus telarius* (L.), does not appear on the apple foliage until late in the season and even then remains very low in numbers. This situation is often very confusing to the grower because he is uncertain as to the need of a definite mite spray program. Thus, when an emergency summer acaricide is indicated for control of the European red mite, it is applied either after most of the damage has occurred or when the population peak has been reached and is naturally declining. In either case, the spray has been costly and ineffective. The development of pre-blossom acaricide sprays within recent years has improved this situation considerably.

Madsen & Borden (1955) found that Mitox® (*p*-chlorobenzyl *p*-chlorophenyl sulfide) and Genite-923® (2,4-dichlorophenyl benzenesulfonate), when applied as a pre-blossom acaricide on pear, held the European red mite in check until July. Oatman (1957) reported season-long control using either Mitox or Genite-923 in the pink stage and ovex in the pink, petal fall, and first cover sprays. Studies in Indiana apple orchards by Cleveland (1958) showed that several of the pre-blossom acaricides were effective for 60 days.

Marshall (1955) believed that mites attacking apples, against which regular control programs are carried out, will become more difficult to control. His prediction has since materialized as reports of European red mite resistance to various acaricides have been received from several areas within the United States and Canada. Madsen & Hoyt (1957) reported resistance to ovex and Genite-923 in California even when applied at twice the normal dosage. Resistance to parathion was reported by Morgan & Anderson (1958) in British Columbia and to parathion and demeton by Cutright (1958) in Ohio. Jeppson, *et al.* (1958) reported that a closely related species, the citrus red mite, *Panonychus citri* (McG.), had become resistant to ovex and 14 organic phosphates including parathion and demeton.

METHODS AND MATERIALS.—The 1956 pre-blossom acaricide control studies were established in a 10 to 12-year-old block of Reynolds Brothers' orchard. Plots consisted of single-tree treatments replicated three times (two on Red Delicious and one on McIntosh). The 1957 pre-blossom acaricide control studies were established in a 12-year-old block of McIntosh and Cortland apples in the Peterson orchard. Treatments were applied to single blocks four rows wide and $\frac{1}{4}$ mile long. The check (unsprayed) was two rows wide and $\frac{1}{4}$ mile long. The 1957 summer-foliage acaricide sprays were applied to a different area within the Peterson orchard. Plots consisted of single-tree treatments replicated three times (two on Cortland and one on McIntosh).

The 1956 pre-blossom and the 1957 summer-foliage acaricide single-tree treatments were applied by an orchard hand gun from a conventional 35 g.p.m. high pressure sprayer operating at 600 p.s.i. The trees were thoroughly sprayed from inside out to the point of drip. The 1957 pre-blossom acaricide single-block treatments

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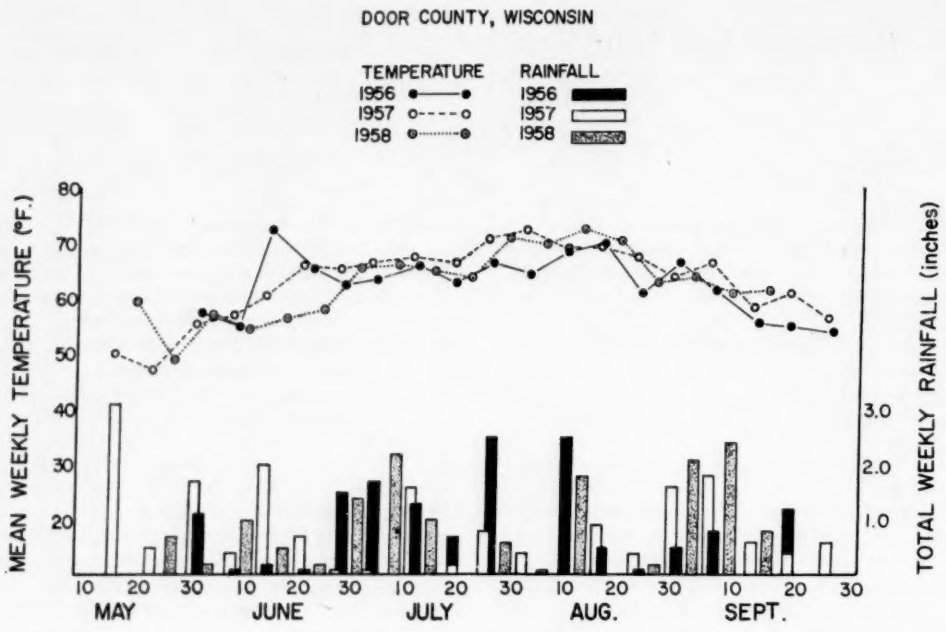

FIG. 1.—Mean weekly temperature and total weekly rainfall for Door County, Wisconsin, during the growing seasons of 1956, 1957, and 1958.

were applied with the owner's 35 g.p.m. high pressure sprayer (with air blast attachment) operating at 600 p.s.i. Sprays were applied thoroughly by driving into the wind so that any drift present would fall back upon the same row.

Materials applied and rates and time of application for both the pre-blossom and summer-foliage acaricide control studies are shown in table 1.

The mite populations were followed throughout each growing season by collecting weekly samples of 100 mature leaves per treatment and check. In the 1956 pre-blossom and 1957 summer-foliage acaricide studies, 33 leaves were sampled from 2 trees and 34 leaves from the third per treatment and check ($\frac{2}{3}$ from outside and $\frac{1}{3}$ from inside). In the 1957 pre-blossom studies 25 leaves were sampled at random from each of 4 McIntosh trees per treatment and check ($\frac{3}{4}$ from outside and $\frac{1}{4}$ from inside). Leaf samples were placed in pliofilm bags and immediately taken into the laboratory where they were brushed with a Henderson mite brushing machine (Henderson & McBurnie 1943) onto vaseline-coated glass counting disks similar to those described by Morgan *et al.* (1955). All active forms and eggs were counted with the use of a stereoscopic binocular microscope. Predaceous mites were also recorded.

Table 1.—Rates and application times of acaricides tested for European red mite control on apples.

ACARICIDE	RATE/100 GALLONS	TIME OF APPLICATION (GROWTH STAGE)
Pre-blossom sprays—1956, 1957		
50% Genite, EC	1.5 pts.	Pink
40% Mitox, WP	1 lb.	Pink
50% Ovex, WP	0.25 lb.	Pink, calyx & first cover
Summer foliage sprays—1957		
25% Chlorobenzilate, WP[a]	1.5 lbs.	Cover
40% Mitox, WP	1 lb.	Cover
15% Aramite, WP	1.5 lbs.	Cover
25% Kelthane, WP[b]	1.5 lbs.	Cover
28.5% Demeton, EC	0.75 pt.	Cover

[a] Ethyl 4,4'-dichlorobenzilate.
[b] 1 1-bis(p-chlorophenyl)-2,2,2-trichloroethanol.

RESULTS AND DISCUSSION.—*Control Studies.*—The results of the pre-blossom acaricide control studies show that all three materials gave season-long control in both the 1956 replicated single-tree plots and in the 1957 large nonreplicated block treatments (table 2). Genite provided superior control in both years. Over the 2 years, Mitox gave better control than ovex although ovex maintained the mite population at a lower level in 1956. Mitox compared favorably with Genite in the grower cooperative tests of 1957.

The results of the summer-foliar acaricide control studies show that although the sprays were applied when the mite population was declining naturally, all materials gave a substantial reduction in the population over that of the check, with demeton giving immediate and lasting control (table 3). Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) gave a quick reduction but control was of shorter duration than that provided by the others.

The results indicate that pre-blossom acaricides will

The 1958 European red mite population studies were initiated following the loss of the crop in the Peterson Orchard caused by spring frosts. No sprays were applied throughout 1958 in the part of the orchard that was the site of the 1957 acaricide studies. Weekly samples were taken during 1958 from the same McIntosh trees sampled during 1957. The sampling and counting techniques were the same as those described for the 1957 pre-blossom acaricide studies.

Table 2.—Effectiveness of pre-blossom acaricide sprays against the European red mite, 1956 and 1957.

Survey Dates	Number of Active Forms per 100 Leaves[a]			
	Genite—923	Mitox	Ovex	Check
1956[a]				
June 13	1	0	1	28
20	0	1	0	252
27	0	0	0	684
July 3	0	1	6	436
11	5	4	10	768
18	11	5	46	838
25	12	33	9	2,612
Aug. 1	60	236	120	3,025
8	136	56	232	1,460
15	632	492	596	1,150
22	704	1,024	772	931
29	608	188	480	712
Sept. 5	344	460	408	124
13	238	534	220	98
19	224	468	208	48
Oct. 1	96	376	128	60
1957[b]				
May 22	48	130	92	178
30	2	8	8	410
June 6	0	0	6	118
12	0	0	2	18
19	0	2	2	504
26	0	2	4	1,294
July 3	0	0	0	616
10	0	0	2	706
18	0	0	18	2,226
24	2	0	6	2,214
31	4	0	20	1,128
Aug. 8	1	2	50	1,846
14	2	4	44	916
21	14	4	28	248
28	4	28	190	340
Sept. 4	44	76	406	298
12	50	88	652	156
19	36	124	608	134
26	74	134	268	36

[a] Pre-treatment count averaged 78 mites per 100 leaves on May 31.
[b] Pretreatment count was 552 mites per 100 leaves on May 15 (400 leaves sampled).

Table 3.—Effectiveness of summer-foliar acaricide sprays against the European red mite, 1957.

Acaricide	Number of Active Forms per 100 Leaves[a]				
	August			September	
	16	21	28	4	12
Chlorobenzilate	123	22	100	42	38
Mitox	181	70	22	0	0
Aramite	35	184	410	122	52
Kelthane	210	218	168	8	2
Demeton	83	54	18	2	0
Check	422	602	578	234	110

[a] Pre-treatment count was 197 mites per 100 leaves on Aug. 12.

give season-long control of the European red mite under the normal seasonal weather conditions which prevail in Door County, Wisconsin. Where pre-blossom acaricides are not used, there are numerous summer-foliar acaricides available to the grower that will give good control with one application if properly timed and thoroughly applied.

Population Studies.—The population growth curve of the European red mite's active and egg stages for 1956, 1957, and 1958 is shown in figure 2. There were five generations in 1956 and 1957 and four generations in 1958. The last generation was always very low in numbers. In general, the population reached a peak the last week in July and then began an abrupt and natural decline during

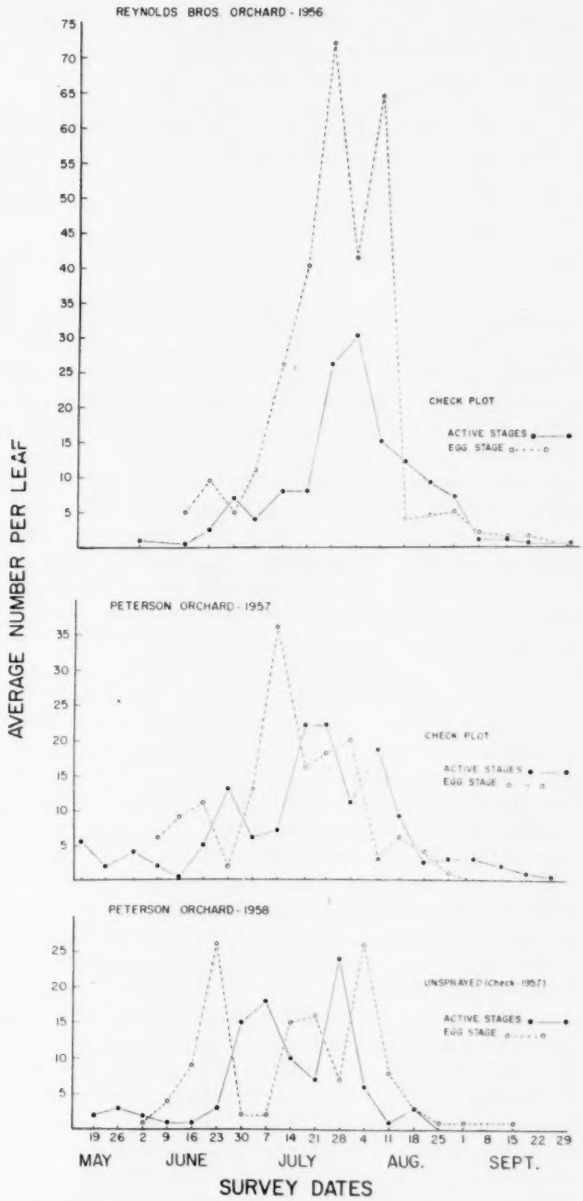

FIG. 2.—European red mite population growth curves for 1956, 1957, and 1958 (eggs at zero on survey date previous to their appearance as shown on graph).

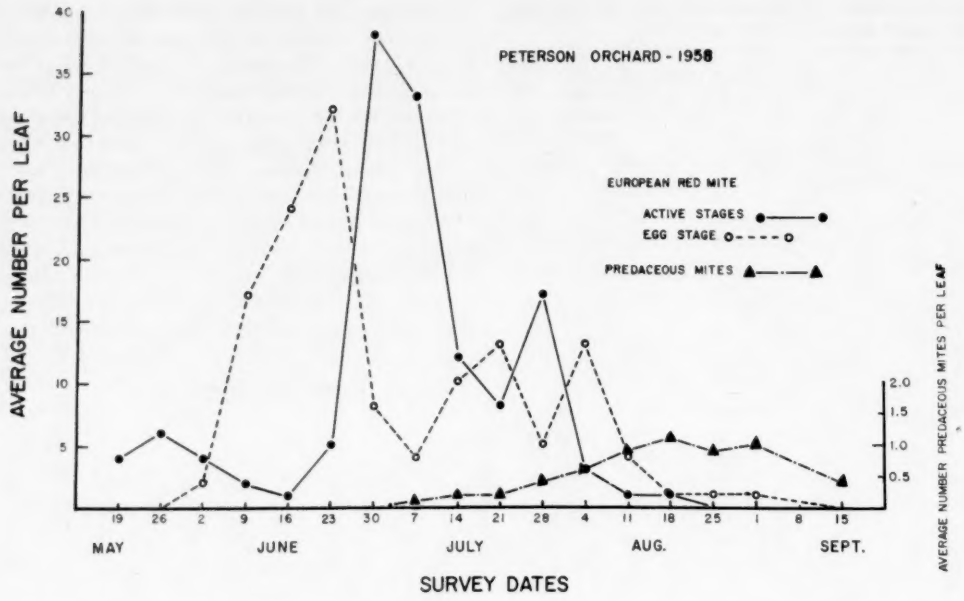

FIG. 3.—European red and predaceous mite population growth curves in an unsprayed orchard in 1958.

the first week in August. The decline in numbers was correlated with the deposition of winter eggs beginning the first week in August. Morgan & Anderson (1958) stated that the overwintering eggs were laid by the first of August in British Columbia. Parent & Beaulieu (1957) working in Southwest Quebec reported that the overwintering eggs were laid between mid-August and early October and hatched out about flowering time each spring. There were six to seven generations per year in that area. Cagle (1946) stated that 10 generations per year occurred in Virginia with the overwintering eggs being laid in the last part of August and hatching during the latter part of the following April and the first week in May. The overwintering eggs began hatching in Door County orchards about the pink stage development of the trees, which occurred the last week in May in 1956 and the second week in May in 1957 and 1958. The first summer-generation eggs were laid by blossom time each year (first week in June 1956 and last week in May for 1957 and 1958) whereas Cagle (1946) reported that the first summer eggs were laid the second week in May. Peaks of active and egg stages were clearly defined with population growth curves of the egg stage following that of the active stages. Lines, representing the growth curves of each, crossed at more or less regular intervals. Such was not true, however, in 1956 when an overlapping of the third and fourth generation of the active forms was apparent. Also, the second and third generation egg stages reached a higher peak and remained at higher numbers than the active stages. The late spring of 1956, which was followed by unseasonably high temperatures in late June (fig. 1), could have provided very favorable conditions for maximum production of eggs by the first two summer generation adults. The peak of eggs shown for 1956 is well within the potential of the adults. Cagle (1946) reported an average of 18.8 eggs per female. The highest peaks of eggs in 1956 and 1957 were those laid by the first summer generation adults. This was not true, however, in 1958 (fig. 2). Anderson (1947) stated that population buildup depends primarily on favorable weather in the spring, the most critical period being from the hatching of the overwintering eggs to the deposition of the first summer eggs. He also reported that only 7 days were required for development from egg to adult at 70° F. Cagle (1946) found that development from larvae to adults required 20 days at 55° F., 15 days at 60° F., and only 10 days at 65° F. The incubation period of the egg required 5 days at 76° F. as compared with 20 days at 56° F. The population growth curves (fig. 2), when related to the prevailing temperatures (fig. 1), appear to agree with these data.

Figure 3 shows the population growth curve for 1958. This result is a composite of the four curves shown in figure 5. Four distinct generations are shown with some indication of a fifth. It is possible that the predaceous mite populations (fig. 3) depressed what would have normally been the fifth generation. A fifth generation did occur the latter part of August in 1956 and 1957. The predaceous mites, which surpassed the European red mite population on August 18 and remained at a higher level for the remainder of the season, were sufficient to have brought about natural control at that time. Anderson (1947) noted such a phenomenon in his studies on the fruit tree spider mite in Germany. Predaceous mites were the only predators observed in association with the European red mite throughout the study. At least two species were present. They were *Typhlodromus* (*Amblyseius*) *fallacis* (Garman) and *Typhlodromus* (*Amblyseius*) *finlandicus* (Oudemans).[3] The former species comprised 95% or more of the predaceous mite population in 1958. Predaceous mites were in very low numbers in the check populations in both 1956 and 1957. This was probably caused by repeated applications of chlorinated hydrocarbons in the commer-

[3] Specimens were identified by Dr. D. A. Chant, Belleville, Ontario.

cial orchards, the sites of the studies during those years.

Figure 4 shows the population on the check trees in relation to that on the treated (average of the three pre-blossom acaricide plots) trees. The population on the treated trees reappeared earlier and reached a higher peak in 1956 than in 1957. In both years the population on the treated trees exceeded the population on the check trees by the end of August and continued at a higher level for the remainder of the season.

The population growth curve of each pre-blossom acaricide treatment and check in 1957 is shown in comparison with the population that occurred on the same trees in 1958 when the trees received no sprays (fig. 5). The population in each treated plot exceeded the check population at the end of the 1957 season as in 1956. More mite larvae emerged from the over-wintering eggs in May 1958 on the treated trees than on the check trees. The population in the ovex plots averaged seven mites per leaf by the middle of September, having exceeded the check population during the first week. The mites that emerged on the same trees in 1958 averaged 10 per leaf. The population in the Mitox plots averaged one per leaf, exceeding the check during the last week in September. The active stages emerging the following spring averaged eight per leaf. The population in the Genite plot exceeded the check population the last week in September and averaged ½ mite per leaf. The following spring (1958) they averaged five per leaf. The mites emerging from the overwintering eggs laid on the trees in the 1957 check plot averaged three mites per leaf. In each instance, the 1958 population, following the 1957 treated population, reached its highest peak the last week in June, with peaks higher than the population which occurred on the 1957 check trees. The populations in the check plots in 1956, 1957, 1958 reached their highest peaks the latter part of July.

The results of the 1957 pre-blossom acaricide control studies and the followup 1958 population studies (fig. 5) indicated that more overwintering eggs were laid in the treated plots than in the check. Morgan & Anderson (1958) reported a similar occurrence in relation to their 5-year-control study of the European red mite in British Columbia using parathion. They stated that an abnormal number of overwintering eggs were laid in the parathion-treated plots and that more eggs were laid on trees receiving three sprays than on those receiving two sprays of parathion. Kuenen (1949), in Holland, found that when the fruit tree spider mite populations become high enough to bronze the leaves at high temperatures, they left the

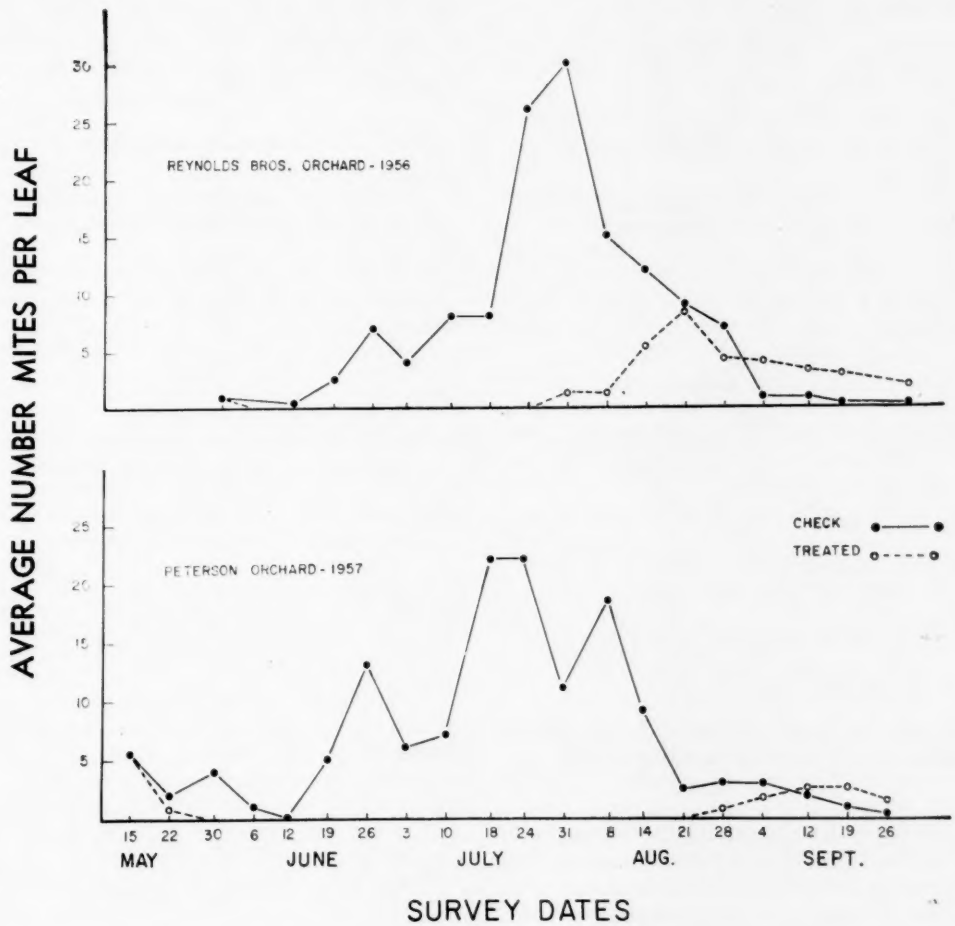

FIG. 4.—Comparison of European red mite population growth curves in check and treated plots for 1956 and 1957.

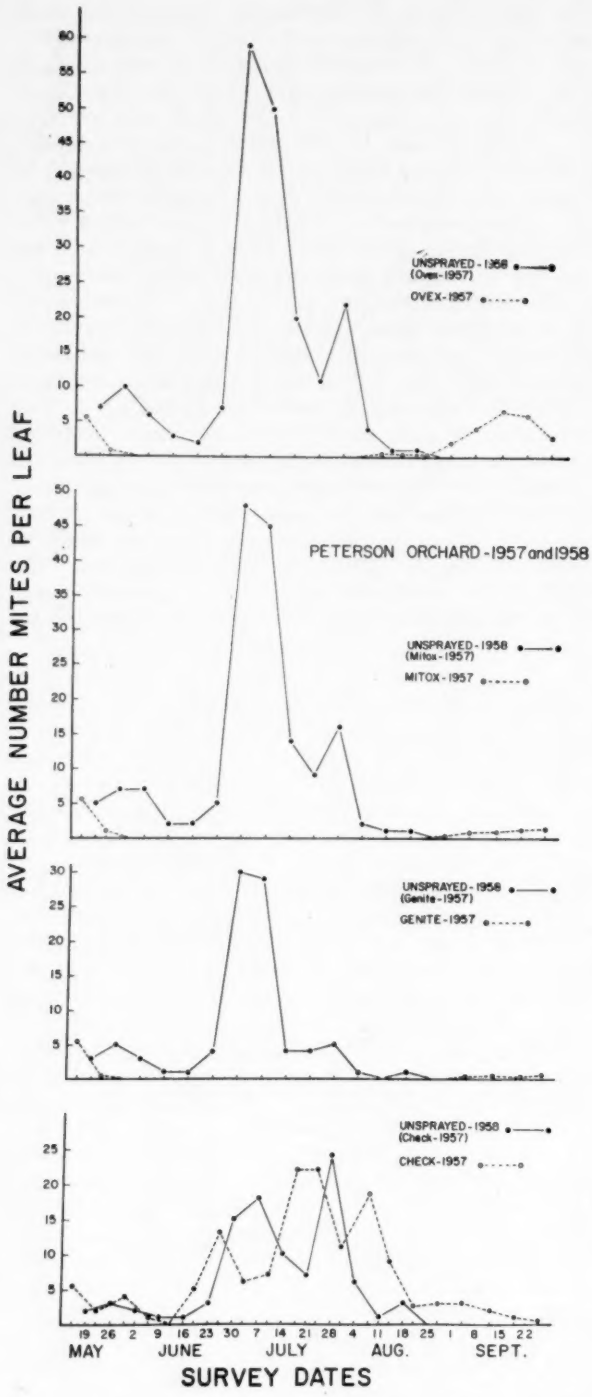
AVERAGE NUMBER MITES PER LEAF
PETERSON ORCHARD - 1957 and 1958
UNSPRAYED - 1958 (Ovex - 1957)
OVEX - 1957
UNSPRAYED - 1958 (Mitox - 1957)
MITOX - 1957
UNSPRAYED - 1958 (Genite - 1957)
GENITE - 1957
UNSPRAYED - 1958 (Check - 1957)
CHECK - 1957
19 26 2 9 16 23 30 7 14 21 28 4 11 18 25 1 8 15 22
MAY JUNE JULY AUG. SEPT.
SURVEY DATES

FIG. 5.—Comparison of European red mite population growth curves in treated plots for 1957 and in unsprayed plots for 1958.

leaves earlier to lay overwintering eggs. They also laid fewer overwintering eggs than where the populations were not high enough to bronze the leaves. Kuenen (1949) reported that populations of medium density that do not overeat their food supply tend to produce very large numbers of overwintering eggs. The present studies in Wisconsin are in agreement with the observations reported previously.

The fact that the low populations on the treated trees late in September 1957 laid unusually large numbers of overwintering eggs lends support to the compensation principle. This principle states, in effect, that one biological phenomenon effects another in order to maintain stability of nature. In this instance the mortality of the species (owing to acaricide) was offset by increased reproduction. Morgan & Anderson (1958) indicated this when they reported that parathion sprays on apple trees over a 5-year period had three different effects on the European red mite: (1) good control initially; (2) gradual development of a resistant strain; and (3) an apparent stimulatory effect. The unusually high and early population peaks (fig. 5) that occurred the last week in June 1958 on those trees treated in 1957 indicated that the compensating factor (or factors) referred to was somehow carried over through the overwintering eggs to the following season's population—in effect, a continuation of the phenomena associated with the compensation principle.

Although the data are not adequate to draw firm conclusions, it does appear that herein lies part of the secret of the European red mite's inherent ability to recover following near complete reduction of the population through adverse environmental conditions caused by toxic chemicals, or its rapid ability to develop resistance to chemical control. Additional basic research into the fundamental biology and ecology of the species is needed to clarify these problems.

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Field Evaluation of Commercially Produced *Bacillus thuringiensis* Berliner Used for Control of Lepidopterous Larvae on Crucifers[1]

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ABSTRACT

Commercially produced samples of spore material of *Bacillus thuringiensis* Berliner were tested in sprays and dusts on cauliflower and cabbage plants for control of cruciferous insects. With coverage an important factor, dusts appeared to be better than sprays. No control of the beet armyworm, *Laphygma exigua* (Hbn.), was obtained. For at least 80% kill of the cabbage looper, *Trichoplusia ni* (Hbn.), and of the imported cabbageworm, *Pieris rapae* (L.), a minimum dosage of 25×10^{12} spores per acre was required. However, it is likely that higher dosage levels of up to 68×10^{12} spores per acre may be necessary for insect control in some situations. The materials were rather short lived on the plants and lost their effect after 5 to 10 days.

Preliminary laboratory and field tests have indicated the susceptibility of various crucifer-feeding lepidopterous larvae to *Bacillus thuringiensis* Berliner and have suggested its possible use in treatment programs designed for the control of these pests on a commercial scale (Tanada 1956, Hall & Dunn 1958). Since the report of these findings, commercial concerns have developed production methods for the mass culture of the bacillus, and an increasing amount of the microbial insecticide has become available for field testing.

The object of this study was to determine the practicability of using this material for control of field populations of the cabbage looper, *Trichoplusia ni* (Hbn.), the imported cabbageworm, *Pieris rapae* (L.), and the beet armyworm, *Laphygma exigua* (Hbn.), on crucifers, and to determine its possible advantages and limitations for commercial usage.

MATERIALS, METHODS, AND RESULTS.—The materials used in these tests were samples of commercially produced *Bacillus thuringiensis* received from Bioferm Corporation, Wasco, California; Merck & Co., Inc., Rahway, New Jersey; and Nutrilite Products, Inc., Buena Park, California. Determinations of potency based upon the number of viable spores per gram of dry material to serve as a guide to the virulence of each product were furnished in most instances by the manufacturers. These counts were checked by germination tests when considered necessary, and each material was rated as to concentration of viable spores. No information was received from the manufacturers concerning the ratio of "crystals" to spores in the different materials, and no determinations of this type were made prior to the field tests, although in most cases the products were proved to be pathogenic to certain test insects in gross bioassay tests. It is known that the insecticidal activity of a crystal-bearing organism such as *B. thuringiensis* is invested in the toxin within the proteinaceous crystal, and the activity of the spore in most instances is considered of little importance against susceptible insects. Therefore, it is recognized that the comparison of the effectiveness of a material from one source with that of another on the basis of only total viable spores is difficult, since materials with the same spore count might vary greatly in crystal content and thus differ strikingly in pathogenicity. However, since no means for establishing the accurate toxic effect of materials other than by bioassay has been developed, it was necessary to rate the various products according to their viable spore counts with the assumption that each material would have a similar ratio of crystals to spores and thus would have a pathogenicity rating comparable to its spore count.

Unless otherwise noted, all dusts were applied with a hand-operated rotary-type duster with a solitary nozzle. Both sides of each row were dusted separately to ensure good coverage. The sprays were applied with a hand sprayer, generally with three flat fan nozzles per row, at 40 to 60 pounds pressure. A suitable wetting agent was added to each material. Insect counts in the test plots were expressed in the number of small (S = first instar), medium (M = second instar to $\frac{1}{2}$ inch in length), and large (L = over $\frac{1}{2}$ inch long) worms alive on a given number of plants.

Test No. 1.—Several bacillus spore preparations were sprayed and dusted on young cauliflower plants 10 to 12 inches tall for control of the cabbage looper and imported cabbageworm. Hollow-cone nozzles were used in applying the initial sprays, but because of clogging difficulties, these were later abandoned in favor of the larger flat-fan nozzles. All plots consisted of single rows of plants and were 100 feet long and replicated four times. The rows treated with dust were bordered by single untreated rows on each side to prevent contamination. The results of this test are presented in table 1.

Despite application difficulties, owing to clogging of the

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Table 1.—The effect of spores of *Bacillus thuringiensis* applied as a spray or as a dust for control of the cabbage looper and the imported cabbageworm on cauliflower plants on the Morimoto farm, Westminster, California, 1958.

Treatment, June 4			No. of Cabbage Loopers[a]/48 Plants After Treatment												No. of Imported Cabbageworms[a]/48 Plants After Treatment											
			June 6 (2 Days)				June 9 (5 Days)				June 16 (12 Days)				June 6 (2 Days)				June 9 (5 Days)				June 16 (12 Days)			
Material (Spores/ (Gm.)	Total Spores/A.	Gals. per A.	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)
Sprays																										
3.33×10^{9}	0.45×10^{12}	48.2	19	15	9	48.2	34	21	4	35.2	—	—	—	[b]	11	7	7	62.1	13	4	4	67.7	—	—	—	[b]
3.33×10^{9}	1.81×10^{12}	51.1	16	10	1	67.5	25	10	6	54.9	3	9	9	32.3	11	9	4	63.6	18	4	2	63.1	4	3	3	37.5
3.33×10^{9}	3.63×10^{12}	47.8	21	10	3	59.0	27	17	1	50.5	0	9	7	48.4	18	9	6	50.0	11	2	4	73.8	1	3	2	62.5
2.5×10^{11}	5.44×10^{12}	23.4	28	10	4	49.4	35	21	5	33.0	—	—	—	[b]	17	16	9	36.4	21	9	8	41.5	—	—	—	[b]
2.5×10^{11}	41.73×10^{12}	42.7	23	7	5	57.8	29	13	6	47.3	—	—	—	[b]	22	7	6	47.0	14	4	5	64.6	—	—	—	[b]
2.5×10^{11}	71.67×10^{12}	37.8	21	7	2	63.9	29	16	3	47.3	8	9	5	29.0	10	7	9	60.6	11	1	2	78.5	3	3	2	50.0
2.5×10^{11}	203.21×10^{12}	53.3	20	10	1	62.7	22	17	2	54.9	2	7	8	45.2	10	5	2	74.2	8	1	1	84.6	1	1	0	87.5
Dust																										
3.33×10^{9}	67.97×10^{12}	45.0[c]	11	3	3	79.5	1	1	0	97.8	2	0	0	93.5	2	4	3	86.4	3	0	1	93.8	0	0	0	100.0
Untreated	—	—	56	25	2	—	56	29	6	—	3	15	13	—	40	10	16	—	31	19	16	—	2	5	9	—
L.S.D. @ 5% level			19.1	7.9	ns		18.2	13.1	ns		ns	ns	6.9		14.7	ns	6.4		9.6	8.9	ns		ns	ns	ns	
@ 1% level			25.9	10.7			24.7	17.7					9.3		20.0		8.7		13.0	12.1						

[a] S = first instar; M = second instar (to ½ inch in length); L = large (over ½ inch in length).
[b] Plots treated with parathion on June 9.
[c] Pounds per acre.

spray nozzles that caused some variation in the number of gallons of spray per acre, certain reliable assertions can be made: (1) although a wide range occurred in the number of spores applied per acre in sprays, the results, based mainly on counts of small and medium worms (since there were not enough large caterpillars to be significant) indicate that in most instances there was not much difference in the per cent control of total larvae of either species of insect. Since some mortality was attained even at the lowest dosages, it was apparent that adequate coverage with the sprayed materials to ensure infection of a high percentage of worms was an important factor; (2) on the basis of total spores per acre, the single dust application gave better control than all the sprays, including the one in which the total number of spores applied was greater. In this case, control was still occurring after 12 days.

On the basis of these observations, it was felt that the coverage of the material on the plant was extremely important and that perhaps the better control by the dust was caused by a more thorough coating of the bacillus material being deposited over the plant (especially on the underleaf surfaces where the smaller- and medium-sized larvae occur). Therefore, it was decided to work with dusts to establish the dosage level (spores per acre) most suitable for control of insects on crucifers.

Test No. 2.—Four spore dust treatments, in which the manufactured dust (3.33×10^{9} spores per gram) was formulated with pyrophyllite to the desired spore counts, were applied to young cauliflower plants 8 to 9 inches tall for control of the cabbage looper. Each plot was three rows wide and 75 feet long and was replicated four times. The results of this test appear in table 2.

The differences in density, owing to the varying amounts of pyrophyllite, appeared to affect the flowability of the materials. However, a comparison of the total number of spores applied per acre with the worm counts suggests a direct dosage-mortality response. It will be noted that after 2 days the greatest mortality occurred with the smaller worms, while after 6 days there was a significant reduction in the number of cabbage loopers of all sizes. At these dosages, the material seemed to lose its effectiveness sometime between 6 and 12 days.

A small number of imported cabbageworms also was

Table 2.—Effect of *Bacillus thuringiensis* applied as a spore dust to cauliflower plants for control of the cabbage looper on the Wada farm, Westminster, California, 1958.

Treatment,[a] June 24		No. of Cabbage Loopers[b]/80 Plants After Treatment											
		June 26 (2 Days)				June 30 (6 Days)				July 7 (12 Days)			
Material (Spores/ Gm.)	Total Spores/A.	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)
3.33×10^{9}	36.45×10^{12}	9	40	10	68.6	8	5	2	91.1	10	21	6	54.9
1.11×10^{9}	12.15×10^{12}	11	48	10	63.3	24	19	5	71.6	5	29	8	48.8
5.55×10^{8}	6.07×10^{12}	18	66	8	51.1	18	34	11	62.7	5	32	19	31.7
5.00×10^{6}	0.05×10^{12}	35	88	14	27.1	28	53	33	32.5	13	37	34	0.0
Untreated	—	43	116	29	—	38	91	40	—	9	36	37	—
L.S.D. @ 5% level		18.9	29.3	ns		16.0	16.1	19.2		ns	ns	ns	
@ 1% level		26.1	39.7			22.2	22.2	26.5					

[a] Spore dust applied at the rate of 24.13 pounds per acre.
[b] S = first instar; M = second instar (to ½ inch in length); L = large (over ½ inch in length).

Table 3.—Effect of *Bacillus thuringiensis* applied as a spray or dust to cauliflower for control of the cabbage looper on the Wada farm, Westminster, California, 1958.

Treatment, July 2			No. of Cabbage Loopers[a]/80 Plants on July 7 (5 Days After Treatment)			
Material (Spores/Gm.)	Total Spores/A.	Lb. Dust or Gal. Spray per A.	S	M	L	Control of Total Worms (%)
Dust						
1.66×10^{9}	24.99×10^{12}	33.2	2	10	3	79.5
1.11×10^{9}	22.66×10^{12}	45.0	0	6	6	83.6
Spray						
2.00×10^{11}	85.28×10^{12}	94.0	6	42	18	9.6
Untreated	—	—	9.3	33.5	30.2	—
L.S.D. @ 5% level			ns	19.0	22.8	
@ 1% level				27.3		

[a] S = first instar; M = second instar (to ½ inch in length); L = large (over ½ inch in length).

present in the field. Although the counts are not presented in table 2, they revealed that the cabbageworm population followed much the same trend as the population of cabbage loopers in response to the dosage applications. On the last count, the cabbageworms also appeared to be increasing in numbers, thus indicating that the treatments were not long lasting.

Test No. 3.—Two dust treatments and one spray treatment were superimposed over half of each of three of the plots in test No. 2. At this time the plants were 12 to 14 inches high. Each plot was three rows wide and 37.5 feet long and was replicated four times. The results of this test are presented in table 3.

The large size of the plants caused some difficulty in obtaining good underleaf coverage with the spray, although the gallonage applied was relatively high. The mortalities obtained with the two dosages of dust were in fair agreement with the dosage-mortality response obtained in test No. 2 when compared on the basis of total number of spores per acre. In this test, as in test No. 2, the dust applications caused a significant reduction in large and medium-sized cabbage loopers.

Test No. 4.—Effects of three bacillus spore dusts were compared with those of DDT-toxaphene dust on cabbage plants 3 to 4 inches tall and planted two rows to a bed for the control of the cabbage looper, the imported cabbageworm, and the beet armyworm. The dusts were applied with a tractor-mounted rotary, barrel-type duster. A separate nozzle was directed over the center of each row. Each plot was five rows wide and 400 feet long and was replicated four times. The results of this test appear in table 4.

In this test, the small size of the plants made it somewhat easier to obtain good coverage. The commercial application of the bacillus dust mixture caused considerable mortality to cabbage loopers and imported cabbageworms of all sizes after 5 days, but the effectiveness of the material dropped sharply between the fifth and tenth days. The differences between the dosages were slight and were not significant. The beet armyworm, an insect showing only slight susceptibility to *Bacillus thuringiensis* in laboratory tests (Hall & Dunn 1958), proved to be resistant in the field. The small number of infected larvae noted when the counts were made was reflected in the low mortality figures.

DISCUSSION.—The main objective in this study was to determine the proper dosage of commercially produced *Bacillus thuringiensis* for control of the various major insect pests of cruciferous crops in California. In addition, it was planned to compare the relative effectiveness of the different samples of bacillus preparations. The latter goal was abandoned when the results of the first test indicated that dust applications would be required for satisfactory kill of the pest insects, and only certain of the preparations on hand were adaptable to formulation into dusts.

The superiority of dusts over sprays in this instance lends evidence to the importance of proper plant coverage with the infective material in order to obtain satisfactory kill of the susceptible insects. In this era of revolutionary complex chemical insecticides, many of which have fuming actions that ensure insect mortality, complete coverage has often become of secondary importance. Such is not the case with *B. thuringiensis* because of the mode of action of the organism. The bacillus, with its spores and crystalline inclusions, acts essentially like a stomach poison, of which material the susceptible insect must ingest enough to sicken quickly and die. Therefore, complete or good coverage is essential for significant reduction in population.

The dusts used in these tests were diluted to the desired spore concentrations with pyrophyllite from an original spore material in a celite carrier. The undiluted spore-

Table 4.—Effects of spore dust of *Bacillus thuringiensis* and of toxaphene-DDT dust applied to cabbage plants for control of the cabbage looper, imported cabbageworm, and beet armyworm on the Hauser ranch, Westminster, California, 1958.

Treatment, July 18			No. of Worms[a]/240 Plants After Treatment													
			Cabbage Looper								Imported Cabbageworm				Beet Armyworm			
			July 23 (5 Days)				July 28 (10 Days)				July 23 (5 Days)		July 28 (10 Days)		July 23 (5 Days)		July 28(10 Days)	
Material (Spores/Gm.)	Total Spores/A.	Lbs. Dust/A.	S	M	L	Control of Total Worms (%)	S	M	L	Control of Total Worms (%)	Total Worms	Control (%)	Tota Worms	Contro (%)	Total Worms	Control (%)	Total Worms	Contro (%)
DDT (5%)	—}	32.2	8	8	4	61.5	52	9	3	0.0	4	69.2	9	64.0	15	73.2	17	46.2
Toxaphene (10%)	—}																	
1.11×10^{9}	14.17×10^{12}	28.1	0	2	1	94.2	39	5	0	30.2	2	84.6	9	64.0	51	8.9	38	0.0
2.22×10^{9}	25.64×10^{12}	25.5	3	4	1	84.6	26	20	4	20.6	3	76.9	15	40.0	32	42.9	16	48.4
3.33×10^{9}	36.43×10^{12}	24.1	1	7	1	82.7	45	10	4	6.3	2	84.6	6	76.0	50	10.7	30	3.2
Untreated			13	22	17	—	40	13	10	—	13	—	25	—	56	—	31	—
L.S.D. @ 5% level			6.5	9.5	ns		ns	ns	ns		ns		ns		11.8		ns	
@ 1% level			9.1	13.4											16.5			

[a] S = first instar; M = second instar (to ½ inch in length); L = large (over ½ inch in length).

celite material was not altogether satisfactory as a dust because of poor flowability through the duster ducts and a tendency to drift when applied rather than impinge on the plant surfaces. Although good kill of the cabbage loopers and the imported cabbageworms was obtained with the dilutions used in the tests, it is believed that effective control with lower dosages may be possible with the use of better dust formulations prepared by the commercial concerns. In this regard, it is noteworthy that if the material has too low a spore count, the increase in the bulk of the material may have an adverse effect on its application.

The standardization of dosages is more difficult with a material like *Bacillus thuringiensis* than with a chemical insecticide. The commercial microbial material contains two ingredients—viable spores and crystalline inclusions. While the latter are known to be the more important, since they create a toxic effect in susceptible insects and are theoretically present in unaltered material at the same concentration as the spores, their role as a separate insecticidal entity has not as yet been exploited. The materials have been standardized only on the number of viable spores present in each gram of dry material and the several commercial concerns attempting production of the bacillus have stabilized on experimental products with no effort at establishment of an industry-wide uniformity of spore (or crystal) concentration. It is evident that a more effective method of standardization is necessary for understanding and use of *B. thuringiensis* by the experimental worker and the practicing entomologist. Although the results reported in this paper are expressed in terms of total spores per acre, the authors found it prudent to select arbitrarily a standard concentration for use in their field studies. The choice of a level of 100 billion spores per gram (with an assumed equal concentration of crystals) permitted easy conversion of any less-concentrated or more-concentrated material to a level that would allow dosages to be expressed in terms of ounces or pounds (rather than fractions or multiples thereof) of material per acre.

It is apparent that the dosage of *Bacillus thuringiensis* most suitable for control of the cabbage looper and the imported cabbageworm varies with the worm population, larval size, and size of the plants being treated. It appears that dusts are much more effective than sprays in achieving the coverage necessary to assure good kill of the pest insects. The results of the tests with the various samples of commercially produced bacillus material indicate that a minimum dosage of about 25×10^{12} spores per acre may be needed to obtain at least 80% kill of the cabbage looper and the imported cabbageworm. Moreover, it is likely that dosages up to the level of 68×10^{12} spores per acre may be required in some situations to ensure effective control of the insects. In terms of the 100 billion spores per gram level of standardization as used by the authors, the above dosage range would be expressed as about 0.5 to 1.5 pounds of 10×10^{10} spores per gram *B. thuringiensis* applied in about 30 pounds of total dust per acre. In losing effect after 5 to 10 days, the bacillus material does not appear to be long lasting on crucifers, and repeated applications may be necessary.

Since the bacillus is not highly pathogenic to some insects, such as the beet armyworm, that may appear on cabbage or cauliflower and has no effect on the cabbage aphid, it is evident that on occasion, where the bacillus is used, there may be a need for supplemental applications of chemical insecticides or other as yet undeveloped microbial materials.

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Pseudococcus citriculus Green and Its Parasites[1]

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ABSTRACT

The citrus-infesting mealybug *Pseudococcus citriculus* Green has frequently been misidentified as *P. comstocki* (Kuw.), a species not known to reproduce on citrus. *P. citriculus* is widely distributed in East Asia and appears in certain regions to be held at low densities by entomophagous insects. Early importations into California of the parasites of this mealybug were released for the control of *Planococcus citri* (Risso). The life history of *Coccophagus pseudococci* Comp., like that of *C. gurneyi* Comp. parasitic in *Pseudococcus gahani* Green, is peculiar in that the unfertilized egg hatches only when the body fluids of the parasitized mealybug are consumed by a companion parasite. *Pseudococcus citriculus* parasitized by *Coccophagus pseudococci* has been obtained from citrus and *Pandanus* but only in the vicinity of Taipo Market in South China. The continuing successful control of *P. citriculus* on citrus in Palestine by *Clausenia purpurea* Ishii may be dependent on the absence of the hyperparasite *Lygocerus* sp.

During the past 25 years a citrus-infesting mealybug has been confused with *Pseudococcus comstocki* (Kuw.), a species which it closely resembles in appearance and in structure (Ferris 1950). Specimens of the citrus-infesting species collected in China, Formosa, and Brazil were recently reexamined by Howard L. McKenzie, University of California at Davis, and identified as *P. citriculus* Green. Most, if not all, specimens of the so-called *P.*

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comstocki collected on citrus are to be considered *P. citriculus*.

Green (1922) described this species from material collected in 1910 from citrus in Ceylon. A survey of the literature shows that the name *Pseudococcus citriculus* has been used to designate this mealybug only on three occasions, Ehrhorn (1932) noting the discovery in 1931 of this species on citrus in Hawaii, Clausen (1933) stating that in Singapore and Kuala Lumpur it was occasionally abundant, and Borkhsenius (1948) correcting its misidentification on citrus in Palestine. It is noteworthy that in 1932 S. I. Kuwana (Compere 1933) considered that the common citrus-infesting mealybugs in Japan were undescribed.

The distinction between *Pseudococcus comstocki*, which feeds on deciduous plants, and the citrus-infesting *P. citriculus* is emphasized by the fact that the serphoid *Allotropa burrelli* Mues. will parasitize the one but not the other. This parasite was reared from mealybugs taken in the field on pears but not from any taken on citrus (Rivnay 1946). This difference in parasitization was confirmed by Rivnay in laboratory tests.

The mealybug parasite *Coccophagus pseudococci* Comp. has been reared several times from the citrus-infesting mealybug but none at all from the form on deciduous fruits (Compere 1933, Flanders 1953, Smith & Flanders 1950).

Silvestri (1930), while exploring East Asia in 1924–25 for natural enemies of citrus pests, noted that *Pseudococcus citriculus*, although the most common citrus-infesting mealybug throughout the various provinces of China, was temporarily abundant only when "protected" by strong colonies of the ant *Polyrhachis sp.* He also noted that the screw palm, *Pandanus*, growing along the shores of Tolo Harbour near Taipo Market was a host of a mealybug similar to *Pseudococcus comstocki* and that this mealybug was parasitized by a *Coccophagus* (Harry S. Smith correspondence). *Pandanus* had been previously reported as a host of this mealybug in Samoa by Laing (1927). In 1954 the writer collected from *Pandanus odoratisimus*, growing near Taipo Market, a mealybug which appeared to be *Pseudococcus citriculus*. It is noteworthy that this material may have been collected from the patch of *Pandanus* from which Silvestri collected his specimens. In any case, this mealybug was also parasitized by *Coccophagus* which Harold Compere identified as *pseudococci* Comp., a species which he had described from specimens that he had reared out of *P. citriculus* taken on citrus near Taipo Market (Compere 1933).

Clausen (1933) noted that *Pseudococcus citriculus* in Malaya, Java, and Sumatra is less injurious than *Planococcus citri* (Risso) because it lacks the habit of congregating in masses on the fruit. On pummelo, its preferred host, it feeds on the smaller branches and undersurfaces of the leaves. Repeated collections during November and December 1932 of *P. citriculus* from citrus in China by Compere (1933) showed no large colonies of the mealybug except on several small branches of three small trees in a citrus orchard in New Territories, Hong Kong. Predators and parasites by the end of December had almost entirely destroyed these large colonies in spite of the fact that ants swarmed over the infested twigs. In China, according to Compere, the conditions under which the mealybug exists warrants the assumption that a satisfactory degree of natural control is maintained by natural enemies, the mealybugs being confined mostly to the inner surfaces of leaves matted together or otherwise protected. *P. citriculus* exists in Brazil under similar conditions. Extensive and intensive inspection of citrus in the state of Sao Paulo by the writer in March and April 1958 showed *P. citriculus* was generally distributed but scarce. It was found most readily in leaves webbed together by spiders. Many of the mealybugs in such a situation were parasitized.

Compere (1939) reported that citrus trees grown for shade and ornament along the streets of Asuncion, Paraguay, sometimes bear small but exceedingly injurious infestations of *Pseudococcus citriculus*. That such infestations are ultimately destroyed by natural enemies is indicated by the fact that parasitization was evident in the remains of old infestations.

SHIPMENTS OF PARASITES TO CALIFORNIA.—C. P. Clausen, during the years 1916 to 1917, made many shipments to California of citrus-infesting mealybugs collected in Japan (see California Commission of Horticulture insectary notes Nos. 1233, 1289, 1300, and 1316). Clausen recently informed the writer that some of these shipments quite certainly consisted of *Pseudococcus citriculus*. The parasites reared in California from these shipments were species of *Allotropa* and *Clausenia*.

These two species of mealybug parasites were first collected from citrus in Japan in the latter part of 1913 by Smith (1914). At that time the primary consideration of the California State Insectary was the importation of the natural enemies of the citrus mealybug *Planococcus citri*. Japan was a logical place to search for such enemies because there citrus mealybugs were of no importance as pests.

During the winter of 1913–14 several thousand adults of *Allotropa* were released by the Horticultural Commissioners of San Diego and Ventura Counties on citrus trees in their respective counties which were infested with *Planococcus citri*. Beginning January 21, 1914, more than a thousand *Clausenia* were released in Ventura County. Clausen, upon returning to California, released 110 adults of *Clausenia purpurea* Ishii on citrus in the vicinity of Alhambra during the week ending August 9, 1917.

The exact host relation of *Coccophagus pseudococci* was unknown until material was received in California as a result of the Asiatic importation program of the Department of Biological Control of the University of California. In August 1950 adults of *C. pseudococci* (18 females and 3 males) emerged at Riverside from *Pseudococcus citriculus* (McKenzie determination) which had been collected in the citrus orchard at Tong Lok Yuen from which the type specimens of *C. pseudococci* had been obtained.

Laboratory propagation was attempted by placing mated females in a mixed culture of *Pseudococcus maritimus* (Ehr.) and *Planococcus citri*. Surprisingly a generation of females completed their development in a few specimens of *P. citri*. A generation of males was obtained by allowing unmated females of *Coccophagus pseudococci* to oviposit in isolated specimens of *P. citri* that had previously received the eggs of the parasite *Pauridea peregrina* Timb. The male developed as a parasite of *P. peregrina* after the latter had consumed the contents of

the host mealybug. In this respect the life history of *C. pseudococci* is like that of *C. gurneyi* Comp., the males of which are hyperparasites of the mealybug *Pseudococcus gahani* Green (Flanders 1937). However, attempts to produce a second female generation of *C. pseudococci* on pure cultures of *Planococcus citri* failed.

In May 1954 in a temporary laboratory of the University of California at Taipo Market, Hong Kong, a culture of *Pseudococcus citriculus* on *Pandanus* was parasitized by one mated female of *Coccophagus pseudococci*. On May 15, 50 specimens of this mealybug, mummified by *C. pseudococci*, were sent to the Commonwealth Institute of Biological Control at Fontana, California. There these parasitized mealybugs yielded 46 females and 4 males of this parasite. The males unquestionably developed as parasites of the females. All attempts to produce females on pure and mixed cultures of *Pseudococcus maritimus*, *P. gahani*, *P. adonidum* (L.), *Planococcus citri*, *Phenacoccus solani* Ferris, and *Phenacoccus gossypii* T. & C. failed (D. C. Lloyd, *in litt.*).

BIOLOGICAL CONTROL OF P. CITRICULUS IN PALESTINE.—According to Compere (1933) the publication of his observations in China was for the purpose of calling attention to the natural enemies or the citrus-infesting mealybug *Pseudococcus citriculus* in case there should be a necessity to attempt its control by the biological method. Such a necessity was strikingly manifested during 1938 in the citrus orchards of Palestine (Rivnay 1939). In 1940 the polyphagous encyrtid *Clausenia purpurea* was introduced into Palestine from Japan and quickly ended the devastating attack of this mealybug on citrus (Rivnay 1946).

The effectiveness of *Clausenia purpurea* in Palestine is attributed in part to its freedom from direct hyperparasitism. Fortunately, the ceraphronid *Lygocerus* sp., which had parasitized about 50% of the *C. purpurea* pupae imported from Japan, does not as yet occur in Palestine (Rivnay 1946).

The importance of *Lygocerus* as a parasite of *Clausenia purpurea* is revealed by the investigations of Clancy (1944), who stated that in North America it may be the decisive factor limiting the effectiveness of *C. purpurea* in controlling *Pseudococcus comstocki* on deciduous fruit trees. Should *Lygocerus* ever become established in Palestine the importation of *Coccophagus pseudococci* would probably be in order.

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NOTICE TO MEMBERS

Authors are urged to consult the latest revised list of "Common Names of Insecticides." See JOURNAL OF ECONOMIC ENTOMOLOGY 52(2): 361–362. April 1959. Reprints are available free upon request to the Office of the Executive Secretary.

The chemical definition of each insecticide, the name of which does not appear in this list, should be given the first time the name occurs in a manuscript accepted for publication by the Entomological Society of America; also in the abstract.

Effects of Naphthenic and Paraffinic Petroleum Composition at a Comparable Molecular Weight or Viscosity on Photosynthesis of Eureka Lemon Leaves[1]

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ABSTRACT

Experiments were conducted for the purpose of comparing the effects of naphthenic and paraffinic petroleum composition at comparable levels of molecular weight or viscosity on the apparent photosynthesis of Eureka lemon leaves. The procedures followed were the same as those described in a prior report. At a deposit level comparable to that of a practical field dosage, no effects on photosynthesis were evident for the difference in paraffinicity at a molecular weight of 306 to 308 or a viscosity of 70. In the high range of oil deposit, indicative of the situation in oil-soaked tissue, initial inhibition of photosynthesis was about the same, but recovery was faster for plants treated with the naphthenic oil than for those treated with the paraffinic oil of comparable molecular weight or viscosity. The difference in rate of recovery associated with the difference in paraffinicity is greater for oils of comparable viscosity than for those of comparable molecular weight.

The effects of napthenic and paraffinic petroleum composition at a comparable molecular weight on the transpiration of Eureka lemon and Bearss lime plants were compared in a report by Riehl & Wedding (1959b). A brief statement of the background for investigations of the relation of paraffinicity to the effect of petroleum oils on the physiological functions of citrus may be found there.

The experiments reported here were conducted to compare the effects of naphthenic and paraffinic composition of saturated, narrow-cut petroleum fractions at comparable levels of molecular weight or of viscosity on the apparent photosynthesis in Eureka lemon leaves.

MATERIALS AND METHODS.—The oils of naphthenic and paraffinic composition used in these experiments were selected from the series of fractions of parent stocks available in our laboratories by matching for molecular weight or viscosity as nearly as possible. Selected properties of the fractions chosen are listed in table 1. The distillation ranges of the narrow-cut fractions would occur within the distillation ranges of light-medium-grade and medium-grade California foliage spray oils.

Descriptions of the procedures followed may be found in a report by Riehl & Wedding (1959a).

DISCUSSION OF RESULTS.—The data obtained by calculations of regressions of percentage inhibition of photosynthesis on oil deposit for a deposit range of 100 to 300 μg. oil/sq. cm. of leaf surface are listed in table 2, and for a deposit range of 300 to 600, in table 3.

Although the various values of percentage of inhibition listed in table 2 are variable for each of the given intervals after application, the data indicate that the rate of recovery with time is faster for the plants treated with the naphthenic oils. In two instances (table 2), that of oil N-5 at 2 days and of oil P-4 at 21 days, the inhibition is greater than that generally found in work with other oils at the deposit level of 150 μg./sq. cm. (Riehl & Wedding 1959a).

At the moment of application, the deposit of oil occurs on the surface of the leaf; this surface deposit is followed by penetration into the leaf tissue, which is the initial dominant activity of the oil. Dissipation of the oil into the air is also taking place, but at a slower rate than that

Table 1.—Selected properties of the petroleum fractions used.

PROPERTY	PETROLEUM FRACTIONS			
	Naphthenic		Paraffinic	
	N-4	N-5	P-4	P-5
Molecular weight[a]	295	306	308	329
Percentage paraffinic composition				
Lipkin-Kurtz analysis	39.6	41.2	70.5	69.9
Waterman analysis	52.5	54.1	78.7	77.8
Distillation temperature, ° F.				
10%	622	638	655	676
50%	628	642	663	687
90%	633	647	668	694
A.P.I. Gravity	29.4	29.4	37.4	36.6
Viscosity, S.S.U. at 100° F.	70.7	79.5	60.4	69.2
Unsulfonated residue	95.2	94.6	99.0	98.2

[a] Molecular weight determined by viscosity correlations of Mills *et al.* (1946).

Table 2.—Effects of certain naphthenic and paraffinic petroleum fractions on calculated mean percentage inhibition of photosynthesis in leaves of Eureka lemon at intervals after application (deposit of 150 μg./sq. cm. of leaf surface).

PETROLEUM FRACTION	DAYS AFTER APPLICATION	PER CENT INHIBITION OF PHOTOSYNTHESIS	REGRESSION EQUATION, $Y=a+bX$[a]	COEFFICIENT OF CORRELATION, r	F FOR SLOPE AT 1 AND 8 DF
Naphthenic					
N-4	2	1.4	= 0.215X−30.8	0.72	8.85*
	7	− 6.6	= .120X−24.6	.57	3.80
	21	0.1	= −.158X+23.8	−.68	6.98**
N-5	2	21.1	= .136X+ 0.7	.82	16.34**
	7	−10.8	= .203X−41.2	.72	8.49*
	21	−17.4	= .059X−26.2	.03	0.78
Paraffinic					
P-4	2	14.1	= 0.173X−11.9	0.70	7.83*
	7	0.2	= .166X−24.7	.57	3.87
	21	17.7	= .118X+ 0.0	.79	14.04**
P-5	2	1.7	= .199X−28.1	.75	10.49*
	7	5.9	= .257X−32.7	.79	13.06**
	21	9.7	= .167X−15.3	.64	5.45*

[a] Y = Per cent inhibition of photosynthesis; X = μg. oil/sq. cm. of leaf surface.

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Table 3.—Effects of certain naphthenic and paraffinic petroleum fractions on calculated mean per cent inhibition of photosynthesis in leaves of Eureka lemon at a deposit range of 300 to 600 μg./sq. cm. of leaf surface at intervals after application.

Petroleum Fraction	Days After Application	Per Cent Inhibition of Photosynthesis, μg. Oil/Sq. Cm. 300	500	Regression Equation, $Y=a+bX$[a]	Coefficient of Correlation, r	F for Slope at 1 and 8 df
Naphthenic						
N-4	7	35.5	56.1	$=0.103X+4.6$	0.88	26.17*
	21	−10.8	−2.4	$=.042X-23.4$	.39	1.41
N-5	7	34.4	62.8	$=.142X-8.2$	.79	12.08**
	21	18.7	33.9	$=.076X-4.1$	.65	5.86*
Paraffinic						
P-4	7	32.7	50.3	$=0.088X+6.3$	0.52	2.96
	21	18.8	51.5	$=.164X+30.4$	.89	29.40**
P-5	7	30.4	51.1	$=.083X+9.6$	.64	5.69*
	21	33.1	63.5	$=.152X-12.5$	.88	27.65**

[a] Y = per cent inhibition of photosynthesis; X = μg.oil/sq.cm. of leaf surface.

of penetration. As the oil spreads and penetrates, occlusion of stomata is generally a factor in the inhibition of apparent photosynthesis for the first day or so after application. Variation in these conditions may have entered into the relatively high inhibition for oil N-5 as shown in table 2 for 2 days after treatment. Although the inhibition at 150 μg./sq. cm. in plants treated with oil P-4 is relatively high, the available evidence leads us to the opinion that it probably is within the limits of chance. Real effects on photosynthesis in Eureka lemon leaves are not evident in the data of table 2 for the difference in paraffinicity of the two types of petroleum composition at a molecular weight of 306 to 308 or a viscosity of 70. If oils similar to the fractions shown in table 1 were used for pest control on citrus in southern California, data reported by Riehl & LaDue (1952) indicate that the deposit required would not exceed 150 μg./sq. cm.

In the high range of oil deposit, the data of table 3 indicate that the initial inhibition of photosynthesis is about the same for naphthenic and paraffinic fractions, but that recovery is faster for Eureka lemon plants treated with naphthenic oils than for those treated with paraffinic oils of comparable molecular weight or viscosity. Also, the difference in rate of recovery associated with the difference in paraffinicity is greater for oils of comparable viscosity than for those of comparable molecular weight. In field usage, this consideration would be a factor in oil-soaked leaf tissue. Although the viscosities of oils N-4 and P-5 are comparable, the molecules of fraction P-5 are appreciably larger, as indicated by the molecular weight and the fractional distillation data.

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On the Biology of *Chrysops flavida* in the Yazoo-Mississippi Delta[1]

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Abstract

Several hundred *Chrysops flavida* Wied. larvae were collected in the Delta area of Mississippi in 1955–56. Some were from around the roots of an emergent sedge, others were from around sedge in habitats that were dry for more than 6 months. Adults emerged in the laboratory in every month except August, September, and November. The average pupal period was 5 days. Submerged larvae readily fed on bloodworms in the laboratory but were observed to take no other food. Larvae in the laboratory were alive after being confined below water for 21 days.

In Mississippi's Yazoo-Mississippi Delta *Chrysops flavida* Wied. was a severe pest of livestock for short periods in the springs of 1954 and 1955. Each year populations came to a peak abruptly in May, stayed at a high level for about 10 days, and then declined rapidly. By the last week in June only occasional specimens could be collected, and this population level was maintained, with slight fluctuations, until mid-October.

In 1954 an extremely wet spring and early summer were followed by an extended hot, dry period, which caused many habitats of tabanid larvae to become dry. In the winter of 1954–55, when water was again in the habitats, *flavida* larvae were collected that apparently had survived for several months in dry ground. An opportunity to investigate this phenomenon occurred in 1955–56, when the weather pattern repeated that of the previous year, allowing periodic observations to be made on larvae in two habitats that dried up. Throughout 1955 and the first quarter of 1956 observations were also made on larvae in water-containing habitats, and on field-collected individuals in the laboratory at Stoneville. The combined observations are reported herein.

Since most of the deer fly larvae from the vicinity of Stoneville did not pupate and emerge, and positive specific identification of *Chrysops* larvae cannot be made at this time, some of the larvae reported on may have been other than *flavida*, but judged by adult collections this possibility is slight. Furthermore, in the Delta *Tabanus*

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[2] Lonnie Newsome, Jr., assisted in this investigation.

[3] In cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station.

larvae were almost constantly present in collections of *C. flavida* larvae, and larvae of *Tabanus* and other *Chrysops* species were also found with *flavida* in collections from elsewhere in the State, so that few, if any, of the observations reported in this paper are regarded as being peculiar to *flavida*. The subspecies *C. f. reicherti* Fairchild occurs with *flavida* in the Delta, but since none of the findings presented in this paper are known to apply to one form more than the other, no attempt is made to differentiate between the two.

DISTRIBUTION AND IMPORTANCE.—*Chrysops flavida* is widely distributed in the Eastern United States, and in some areas is the dominant deer fly. Philip (1947, 1950) listed the species from 23 States, the Bahama Islands, Cuba, and Mexico. Brennan (1935) reported collecting records for every month except December and January, and said that other workers, not identified, had reported it from three States not listed in Philip's catalog: Pennsylvania, New Hampshire, and Maine. Including these three States, *flavida* has been collected in every Eastern and Southern seaboard State. Fattig (1946) listed records in Georgia for April, May, June, July, and September. Hine (1906) stated that it was perhaps the most common deer fly on the Louisiana coast in 1906, and the following year (Hine 1907) he collected or observed it in every section of the State visited, and reported that it was the worst livestock pest of its genus. Schwardt (1936) stated that *flavida* was the dominant *Chrysops* species over much of the east-central part of Arkansas.

Snow *et al.* (1957) collected *flavida* from April 18 through September 10, in surveys in the northern Alabama section of the Tennessee Valley watershed, where it was the most pestiferous deer fly observed.

Schwardt (1936), collecting in the rice-growing area of Arkansas, took *flavida* larvae in various stages from around the submerged roots of rice plants. Others were collected from the mud banks of stagnant ponds in northwestern Arkansas. Jones & Bradley (1923) took a larva in mud from under a foot of water in Louisiana.

MacCreary (1940) found that *flavida* larvae were capable of surviving considerable flooding. He collected them around the roots of salt-marsh cordgrass (*Spartina alterniflora*) from under 12 inches of water in a marsh pothole, in the floating sod of salt-meadow cordgrass (*Spartina patens*) from a brackish pool, in floating masses of *Eleocharis parvula* from a slightly brackish pool, and in dilute organic material 200 feet from shore in an area that never changed in moisture content except for an occasional complete flooding. He also collected them from above the water line in ponds and ditches in marshes.

MacCreary called attention to an anomalous form of *flavida*, which was more common than the typical form in the Delaware collections, and Pechuman (1949) later described *C. atlantica* Pech. and at the same time erected the subspecies *C. flavida celata* Pech. from the eastern *flavida* complex. It is not known, however, whether any of MacCreary's anomalous specimens were found to be *atlantica* or *flavida celata*.

METHODS OF COLLECTING LARVAE.—Some of the *flavida* larvae discussed in this paper were taken by exploratory digging or with collecting screens, but most were collected by placing material from the habitats in 2- to 3-inch layers on small-mesh hardware cloth in large Berlese funnels, the sheet-metal tops of which contained two heat lamps with the beams directed on the collected material. When the heating and drying action of the lamps drove the larvae from the media, usually in 24 hours or less, they were caught in pans partially filled with water and lined with paper toweling beneath which the larvae would take refuge. Attempts to collect larvae with a pyrethrum emulsion in the manner described by Bailey (1948) were unsatisfactory.

COLLECTIONS IN APRIL THROUGH JUNE 1955.—From April through June more than 600 *flavida* larvae and pupae were collected. Several were from the mud along the grassy edges of borrow pits near the levee of the Mississippi River in the general vicinity of Greenville. Others were collected on April 25 from a muddy, cattail-bordered stretch of shore line of the artificial lake in Pelahatchie State Park. Several full-grown larvae and one pupa were taken on May 24 from a water-surrounded, grass-covered mound 12 inches in diameter which projected about 8 inches above the surface of a pond near DeLyle. A full-grown larvae was taken on May 28 among the roots of an emergent herbaceous plant in a marshy area bordering the artificial lake in Clarko State Park north of Quitman.

A large number of larvae were collected from a water-filled grader ditch and the cleared, pipeline-traversed portion of a swamp in the Delta Branch Experiment Station forest at Stoneville. Their distribution according to size is presented in table 1. No recently hatched larvae are included, but between mid-May and mid-June 1

Table 1.—Per cent of *Chrysops flavida* larvae of different lengths collected at different times from dried and inundated habitats in the Yazoo-Mississippi Delta, 1955–56.

LENGTH (MM.)	GRADER DITCH AND SWAMP		WATER-FILLED HABITATS AUG. 1 TO MARCH 3[c]
	From Mud or Water April 1 to June 30[a]	From Dry Ground Aug. 1 to Jan. 9[b]	
5– 7	12.7	28.6	0.0
8– 9	11.1	34.0	3.0
10–11	36.8	14.1	9.1
12–16	39.4	23.3	87.9

[a] 244 larvae.
[b] 518 larvae.
[c] 164 larvae.

dishpanful of bottom ooze from the swamp commonly contained as many as 200 of them. Larvae were as far as 100 feet from shore and were concentrated around the roots of the horned rush (*Rhynchospora corniculata* var. *interior* Fern.).[4]

Most of the larvae were taken in April and May from around the artificial lake in Leroy Percy State Park. On April 18 they were found concentrated in drifted leaves near the lake's heavily tree-canopied dam, and around the roots of most herbaceous dicotyledonous plants along the grass-bordered eastern shore line. One dishpanful of leafy material taken immediately above the dam yielded

[4] Determined by W. L. Giles, superintendent of the Delta Branch of the Mississippi Experiment Station.

180 full-grown larvae. Since they were not present in these sites earlier in the spring, the larvae evidently had migrated there just a short time before, when the water level was high. On April 18 the water was starting to subside and all larvae observed in one of the flat areas were adjacent to the water in saturated surroundings. Four days later the water level had receded slightly, but the larvae, still in saturated soil, had not moved. By May 3 the shore line had receded 10 feet, the soil around the insects was damp but not muddy, and approximately a third of all observed had pupated. By May 10 the water line had declined 30 feet, most individuals had pupated, and the many empty pupal cases indicated that emergence was near its peak. One week later empty pupal cases were abundant but larvae or pupae could be found only with difficulty. During the next month diligent searching along the east shore yielded only newly hatched larvae.

COLLECTIONS FROM WATER-FILLED HABITATS AFTER AUGUST 11.—Most larvae collected from water-filled habitats after the first of August (table 1) were from around the roots of emergent horned rushes, cattails, or other herbaceous plants, and all were beneath or adjacent to water. These habitats included grader ditches, the grass-bordered shores of borrow pits near the Mississippi River, and the lake's edge in Leroy Percy State Park. No larvae were found in drifted leaves, as was the case at pupation time in the spring. Most of them were collected in routine sampling and are regarded as representative of the large populations in the areas.

In a search for larvae in habitats without herbaceous plants, 3 half-days were spent screening bottom samples 5 to 25 feet from shore above the dam in Leroy Percy Lake in an area that contains emergent trees and shrubs but no emergent herbaceous vegetation. No larvae were found, although they could be collected from around horned rushes a short distance away. Extensive digging with a hand pick in September and October in the dried-up, tree-covered portion of the swamp in the forest at Stoneville yielded no larvae, although they were abundant among the roots of horned rushes on the adjacent herbaceous-covered pipeline right-of-way. The hot, dry weather at this time caused the water level in several habitats to fall below normal. In contrast to their distribution in the spring, *flavida* larvae were rarely found in samples from the roots of plants left above the receding water.

From August 1, 1955, through March 3, 1956, frequent checks were made on the heavy larval populations in a water-filled grader ditch north of Stoneville and in Leroy Percy Lake. Except for a few days in September, during which water almost disappeared from the grader ditch, the water level in both habitats was fairly constant throughout the period. Although most of the larvae in both habitats were full grown by August 1, they did not migrate to drier ground where they could pupate, and pupae or empty pupal cases were not found in either area.

LARVAE FROM TWO DRIED-UP HABITATS.—A grader ditch in the station forest at Stoneville, where the larval population was under surveillance, dried up in mid-June, and a section of the swamp that was cleared for a pipeline right-of-way in the same forest dried up about July 1. As the standing water in the two areas disappeared, all larvae below the upper 1½ inches of soil became quiescent and the black Sharkey clay of the habitats, which shrinks as it dries, contracted firmly around them.

Table 2.—Depth from which *Chrysops flavida* larvae were collected in dried-up habitats at Stoneville, Mississippi., in 1955.

Date Collected	Number	
	0–4 Inches	4–10 Inches
October 28	27	5
October 31	28	9
November 7	6	0
November 9	14	1
Total	75	15

By September 1 the cleared portion of the swamp was dry and firm enough to support a car. During this dry period, which continued until mid-January, 518 larvae were collected from the two areas. Some larvae were taken by exploratory digging, but most were collected in circular soil samples 14 inches in diameter by 6 inches deep from around the roots of horned rushes. Maximum larval recovery was obtained by soaking the samples in lukewarm water from 12 to 24 hours, crumbling them finely, and placing them in Berlese funnels. Less than one-fourth of the larvae taken from dry ground were full grown (table 1), but most of those from water-filled habitats during the same period were full grown. Depth samples revealed that most larvae were between 1½ and 4 inches deep, but only a few were below 6 inches (table 2).

When the standing water disappeared in the two habitats, the upper 1½ inches of soil became powder dry and no larvae survived in this soil fraction, but it did contain empty pupal cases. Since pupae of this species cannot survive under water and a pupa was found in an adjacent similar habitat that had recently dried up, the empty pupal cases apparently represented larvae that had pupated and emerged as the soil was drying.

On January 9, 1956, the final collection from the dry ground contained 6 larvae that were full grown, 24 that were between 8 and 11 mm. in length, and 61 that were less than 8 mm. During the half-year period of quiescence no measurable reduction had occurred in the population below the 1½-inch level. Soon after January 9 both habitats were again inundated.

LABORATORY OBSERVATIONS ON FIELD-COLLECTED INDIVIDUALS.—During 1955 and the first 3 months of 1956, 368 *flavida* adults emerged in the laboratory at Stoneville (table 3), of which 186 were males. The shortest pupal period observed was 3 days (1.7%), the longest 7 days (6.7%), with the greatest emergence (59.4%) on the fifth day. However, Schwardt (1936) reported a pupal period as long as 9 days for the species, and one field-collected pupa at Stoneville was held for 9 days before it emerged. Limited observations indicated a prepupal period of 1 day.

Approximately 92% of the emergence in the rearing room occurred in April and May (table 3), but some pupation occurred in every month except August, September, and November. Prior to mid-June the normal temperature in the rearing room was 80° ± 2° F., but it sometimes rose higher. From mid-June to December 1 an air conditioner held the maximum to 76°, and during

Table 3.—Monthly emergence records of 368 *Chrysops flavida* individuals.[a]

MONTH	MALES	FEMALES
January	5	4
February	2	2
March	1	2
April	67	26
May	109	136
June	1	5
July	1	1
October	0	3
December	0	3
Total	186	182

[a] No emergence in August, September, or November.

this period only five larvae pupated. Since pupation and emergence occur in nature until mid-October in the vicinity of Stoneville, and an ample supply of full-grown larvae was present in the rearing room at all times, this temperature apparently is near the minimum that will stimulate pupation during this period. Temperature probably is the primary factor in stimulating pupation, for when eight full-grown larvae, collected on December 13 from beneath ice in a water-filled grader ditch, were held in shell vials in contact with moist paper toweling at 80° ± 2°, pupation occurred on December 31, January 1, 5, 6, 15, 28, and February 4 and 27.

Larvae 12 mm. long were observed to pupate, but the average full-grown larva is longer, and the maximum length is 18 mm. where measurements are based on the fully extended larvae as they crawl in water. No other method of measuring larvae was consistently repeatable. The wide range in size of full-grown larvae is illustrated by the weights of 12 randomly selected specimens which ranged from 15.7 to 46.2 mg. and averaged 28.8 mg. Pupal sizes also vary widely.

When quiescent larvae from dried-up habitats were placed in contact with moist paper toweling, there was usually a period of persistent activity which subsided gradually. During this active period weight losses occurred, but these losses were recovered when crawling ceased (table 4). Since the larvae were given no food or water for deglutition, the weight gains apparently were from water absorbed through the integument. The data indicate that larvae are capable of storing more water when quiescent than when active. Habitat moisture apparently accounted for the differences in weight between October 6 and November 11.

Feeding Trials.—Many *flavida* larvae were used in feeding trials in the laboratory. Different lots of larvae were offered live and cut-up earthworms, snails, leeches, hamburger, liver, crayfish meat, cooked and raw cream of wheat, strained liver, and various other strained baby-food preparations. Other lots were held in hay infusions of various ages, and in dirt from the habitat to which were added different percentages of moisture, cow or pig dung, or powdered yeast. Measurements on all lots and weighings on some yielded no evidence of growth. Larvae were also placed among the submerged roots of a sprouted sweetpotato, but none were observed to feed on the roots or on the sweetpotato itself. However, they readily fed on bloodworms in water-filled beakers containing a $\frac{1}{4}$-inch layer of sand. The larvae that were observed in the act of feeding were partially within the bloodworms' dwelling tubes, and their feeding motions were indistinguishable from those of *Tabanus* species.

Table 4.—Weight changes of deer fly larvae collected from dry soil and held in contact with moist paper toweling without food.

LARVA NO.	WEIGHT (MILLIGRAMS)				PER CENT GAIN
	Oct. 6	Oct. 7	Oct. 10	Nov. 11	
1	18.7	17.8	18.4	20.2	8.0
2	27.4	26.5	27.4	30.4	10.9
3	29.2	28.5	28.5	32.3	10.6
4	35.0	34.2	34.1	37.8	8.0
5	16.4	15.6	15.7	16.8	2.4
6	5.4	5.0	4.9	5.5	1.8

Submergence Test.—In a submergence test 15 *flavida* larvae of different sizes, together with numerous bloodworms, were confined below the water's surface with a plastic screen for 21 days. Although most of the larvae were active at first and several fed, when the water became fetid, which occurred shortly, they would move only when disturbed. Except for one full-grown and one small larva, all survived for the 21-day period.

DISCUSSION.—The preferred developmental site for *flavida* larvae appeared to be around the roots of emergent herbaceous plants, where tendipedid larvae were present in sufficient numbers to provide food.

Empty pupal cases were found only in dried-up habitats after the first of August, which fact suggests that the small but rather constant population of *flavida* adults that are present after the initial period of massive emergence in the spring or early summer results primarily from pupation that occurs in dried-up habitats. Habitat moisture and temperatures below the $1\frac{1}{2}$-inch level were in ranges favorable for pupation, which suggests that some factor associated with the quiescence, probably the accumulation of metabolic wastes, retarded pupation at the lower depths.

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Spectral Response of Certain Stored-Product Insects to Electromagnetic Radiation[1]

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ABSTRACT

Laboratory studies were conducted to determine the spectral response of seven species of stored-product insects. Nine wavebands of radiation between 280.4 mμ and 600.0 were used for the tests. Four species preferred a waveband which peaked near 500 mμ and one a waveband between 334 to 365 mμ. Another species showed no preference but generally migrated to the light for all wavelengths between 334 and 546 mμ. The seventh species showed insufficient reaction to be conclusive. Additional studies showed that males were generally attracted in greater numbers than females. The reaction of all tested species of insects was increased significantly by an increase in intensity. In general, no change in waveband preference was indicated as the intensity was increased.

Man has been interested from very early times in the fact that insects are attracted to light. In 400 A.D. King Sudraka wrote, "The moth hovers round the wick and extinguishes the fire," suggesting his observance of insects being attracted to light (Frost 1952). Many previous investigations have established the fact that insects have some degree of color perception. Studies conducted by Bertholf (1931), Gui *et al.* (1942), Taylor & Deay (1950), and Glick *et al.* (1955) have indicated that certain insects are more responsive to the shorter wavelengths of the spectrum. Limited research has shown certain stored-product insects were more readily attracted to certain wavebands than to others. Studies by Glick *et al.* (1956) showed that 75 to 94% of the pink bollworm moths (*Pectinophora gossypiella* (Saund.)) collected in traps containing argon lamps were female. Previous studies by Weiss *et al.* (1942) had also indicated that a change in intensity caused a change in spectral response of several species of insects.

Previous research has indicated that the presence of insects at low population levels can be detected by the use of light traps. There is also a possiblity that insect traps using electromagnetic radiation to attract the insects can be used as a criterion for determining the degree of infestation. The spectral response of most of the stored-product insects has not been previously determined. Therefore, research was initiated in the laboratories at College Station, Texas, to determine the spectral response of some of the most injurious stored-product pests, such as the Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) and the rice weevil (*Sitophilus oryza* (L.)). Studies were also conducted to determine the spectral response by sex and the effects of intensity on spectral response. Once the optimum wavelength and intensity have been determined proper evaluation of the light trap as a tool for early detection of insects or for control can be made.

PROPOSED METHOD OF TESTING.—The problem of evaluating the stimulative efficiency of various wavebands to insects requires special attention. The problem is simple for man, since he sees various wave-lengths as different colors and can make known his subjective impressions by speaking. But with insects and other lower forms of life, some physical reflex must be observed in order to evaluate the effect of the light.

Exploratory attempts were made with a variety of methods to determine a procedure suitable for testing the relative response of insects to different wavelengths of radiation. A test chamber measuring 4′×6′×14′ with a radiation source at each end gave the most reproducible results. Therefore, instrumentation was devised for this method of testing. Two independent stable sources of radiation that could be adjusted for wavelength and intensity were installed. The necessary photometric instruments were also secured to calibrate and adjust these sources to the desired condition for the tests.

Previous investigations by other researchers indicate that the spectral region from 350 to 600 mμ is attractive to many species of insects. The literature also indicates a considerable difference in attractiveness of wavebands separated by as little as 30 mμ (Weiss *et al.* 1941). It was necessary, therefore, to develop suitable techniques for producing spectrally pure wavebands less than 20 mμ wide for any portion of the spectral region from 250 to 700 mμ. In addition, a supply of uniformly controlled live insect specimens was required for testing.

TEST EQUIPMENT.—Two Bausch and Lomb[2] grating monochromators with wedge interference filters and appropriate lamps provided a variable wavelength source for each end of the test chamber. A calibrated Type 1P28 multiplier phototube along with associated instrumentation was used to measure the intensity of the radiation. One of the principal difficulties experienced by previous investigators was that of measuring the intensity of monochromatic radiation. This problem was

[1] This paper was endorsed and communicated for publication by Norman M. Dennis, Stored-Product Insects Section, Agricultural Marketing Service, U. S. Department of Agriculture. Accepted for publication March 23, 1959.

[2] Reference to specific instruments or trade names is made for purposes of identification and does not imply endorsement by the U. S. Government.

given careful attention throughout this research. Detailed descriptions of the instrumentation used and calibration procedure employed are described in a previous paper (Stermer 1958a).

As mentioned earlier, a test chamber with dimensions of 4′ wide by 6′ high by 14′ long was found to be most satisfactory. Other test chambers which were used included a 6″×6″×6′ sheet metal tube with a monochromatic light source on one end and a 6″×6″×12′ tube with provisions for a light source at each end. These chambers were of light-tight construction with the interior surfaces painted flat black to eliminate reflected light.

Each end of the final test chamber was equipped with a 4″×4″×16″ fused quartz plate to serve as a window. The plate was ground with 120 grit on both sides so that it acted as a diffuser to provide more uniform radiation over the entire cross-sectional area of the test chamber. The arrangement of the instrumentation is shown in figure 1. The ends of the test chamber were identical. A funnel with provisions for attaching a Mason jar into which a smaller funnel projected was mounted directly below each window inside the test chamber. These funnels trapped most of the insects attracted to the radiation and served as a means of evaluating the response. A commercial type humidifier maintained the humidity at 65% inside the test chamber. The entire test equipment, including the chamber, was operated in air-conditioned laboratory space held at a constant temperature of 80° F.

PROCEDURE.—All insects used in the study were reared under laboratory conditions with all environmental factors, such as temperature, rearing media, et cetera, controlled to eliminate many of the expected variables. Detailed descriptions of rearing and handling techniques employed were reported by Stermer (1958b).

In the small test chambers used in preliminary studies it was necessary to transfer the insects from the rearing jars to holding jars and then to the test chambers prior to testing. This procedure required anesthetizing the moths with carbon dioxide gas and other handling, apparently adversely affecting the insects. The procedure followed in the large test chamber eliminated the handling of the insects prior to testing. This method resulted in less variation and higher significance in results. The only results reported in the present paper are those conducted in the large test chamber.

The test chamber was thoroughly infested with all seven species of insects studied. They were as follows: (1) the rice weevil, (2) the Angoumois grain moth, (3) the almond moth (*Ephestia cautella* (Wlkr.)), (4) the lesser grain borer (*Rhyzopertha dominica* (F.)), (5) the red flour beetle (*Tribolium castaneum* (Hbst.)), (6) the Indian-meal moth (*Plodia interpunctella* (Hbn.)), and (7) the flat grain beetle (*Laemophloeus minutus* (Olivier)). The insects were free to move about in the chamber. Prior to a test, each monochromator was adjusted to a particular waveband as determined by randomization. Nine wavebands from 280.5 to 600 mμ were used to obtain a satisfactory response curve. The intensities on both ends were adjusted to be equal. For the spectral-response studies, day tests were conducted for 3 hours, preceded by a 3-hour period of light. Night tests were conducted for 15 hours, preceded by 3 hours of light. Since the insects studied were negatively phototropic, light conditioning greatly increased the relative response. The insects trapped at each end of the chamber were considered to have been attracted by the radiation. Intensities were checked again at the end of a test to insure that there had been no great change. If the intensity had changed by as much as 10%, the test was repeated. Sufficient replica-

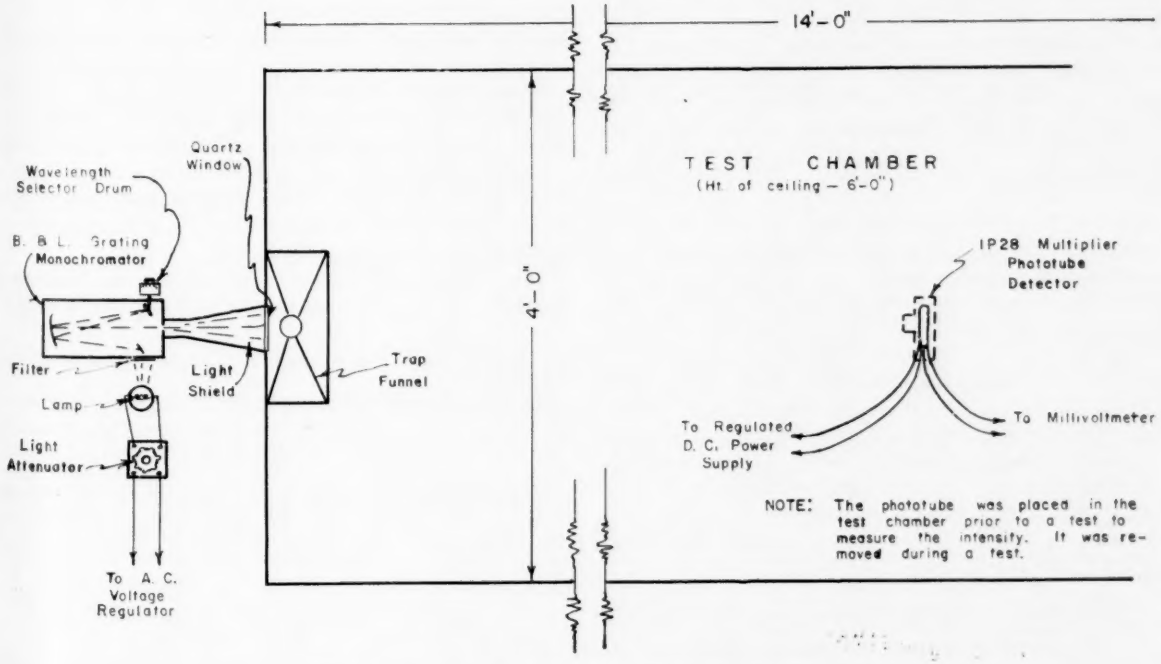

FIG. 1.—Top view of test chamber and related apparatus.

tions were made to give statistically significant results.

Two attractive wavebands (334.1 and 500.0 mμ) and one unattractive waveband (404.7 mμ) were selected as the wavebands to be tested in the sex-response studies. Each of the three wavebands was compared with a reference waveband of 404.7 mμ. The procedure was identical with that of the spectral-response study except that the duration of each test was reduced to 1½ hours. Only the three species of moths were used in this study because of the difficulty in determining the sex of the beetles.

Three wavebands, 365.4, 404.7 and 546.1 mμ, were used in the tests conducted to determine the effects of intensity of spectral response. These wavebands were selected since the spectral-response studies had shown that 365.4 and 546.1 were very attractive, while 404.7 was relatively unattractive. These three particular wavebands were also selected since they are mercury emission lines and therefore produced higher intensities than could be obtained with tungsten sources. Relative intensities of 1, 3, 5, 7, 9, and 11 times normal were used in these studies. Normal intensity refers to the intensity used for the spectral-response studies and was equivalent to approximately 9 micro-microwatts of radiant energy. Test equipment and procedures used were identical with those used in the spectral-response studies except that intensity and wavelength were both varied in accordance with a table of random values. A night- and a day-series of tests was conducted.

The relative response was determined by actually counting and/or sexing the trapped specimens after each test. No killing agent was employed in the traps, since the health of the insects not trapped would have been impaired. The insects were killed by heat in a small electric oven. This killing process, along with the use of excelsior in the jars on the traps, kept the specimens in good condition for identification, sexing, and counting.

RESULTS.—The results of the spectral-response studies showed that four of seven species; the almond moth, the Angoumois grain moth, the lesser grain borer, and the red flour beetle, preferred a waveband which peaked near 500mμ in the green portion of the spectrum. By comparison, the peak response of the average human eye is at 560 mμ. The aforementioned four species selected a waveband between 334 and 365 mμ as their secondary preference. One species, the Indian-meal moth, reversed its preference. The ultraviolet region between 334 and 365 mμ was most attractive to it, while the waveband near 500 mμ was secondary in attractiveness. The rice weevil showed no significant preference for wavebands between 334 and 546 mμ. One species, the flat grain beetle, did not react in sufficient number to show significance. The only apparent explanation for the small response of the flat grain beetle was that many of the specimens escaped from the funnel trap, since they were so small and because no killing agent could be used in the traps. All species tested had a low response to wavebands at 600 mμ and practically no response at 280.4 mμ. A summary of the data is shown in table 1. Each entry is the mean response compared with all other wavebands. Four replications were made, two conducted during the night and two during the day. The data were combined, since no significant difference resulted between night and day tests, and between tests within night and day. Figure 2 shows the response curves for three species of insects compared with the sensitivity of an average human eye.

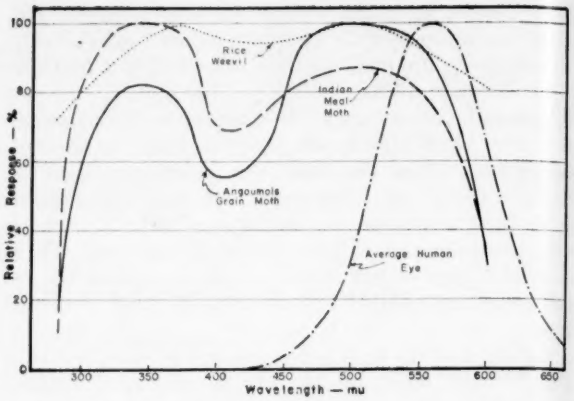

FIG. 2.—Spectral response of three species of insects and the human eye.

The results of studies conducted to determine the response by sex are shown in table 2. More males than females of the almond moth and Angoumois grain moth were attracted to all wavebands tested. No significant difference existed in the percentage of male and female Indian-meal moths reacting to various wavebands or between wavebands and the check.

The reaction of all species of insects was increased significantly by an increase in intensity. The response of

Table 1.—Summary of spectral response studies.

Waveband Tested (mμ)	Mean Response—Per Cent of Insects Reacting Positively to Tested Waveband					
	Almond Moth	Indian-meal Moth	Angoumois Grain Moth	Rice Weevil	Lesser Grain Borer	Red Flour Beetle
280.4	9.0	8.9	13.8	39.4	36.6	22.2
334.1	56.9	68.9	57.5	50.5	48.6	42.6
365.4	57.1	68.7	56.4	54.1	49.9	49.8
404.7	47.9	47.2	39.5	52.3	45.0	46.4
445.0	56.2	54.6	53.2	51.4	53.4	56.2
475.0	61.0	59.7	69.3	53.3	57.0	62.8
500.0	65.3	59.8	70.6	54.5	60.0	66.7
546.1	62.4	57.4	67.6	50.8	60.6	67.5
600.0	36.8	24.9	22.1	44.1	38.8	35.8
L.S.D. at 5% level[a]	3.8	4.3	5.1	6.1	6.2	8.4

[a] The value shown is the difference in mean response required for significance between two adjoining wavebands when the wavebands are arranged in order from most attractive to least attractive. The difference required for significance increases progressively as the wavebands are more widely separated in rank according to attractiveness.

Table 2.—Reaction of male vs. female moths to various wavebands.

Waveband Tested (mμ)	Mean Response—Per Cent Females Reacting to Tested Waveband		
	Almond Moth	Indian-meal Moth	Angoumois Grain Moth
Check[a]	56.2	55.7	54.0
334.1	36.9	49.6	42.1
404.7	32.8	52.4	41.0
500.0	26.4	49.7	39.2
L.S.D. at 5% level	9.4	n.s.	6.6

[a] Check refers to sex population existing in the rearing jars prior to testing.

Table 3.—Summary of response to various intensities.

Waveband Tested (mμ)	Intensity Level	Adjusted Mean Number Reacting Positively to Tested Waveband					
		Almond Moth	Indian-meal Moth	Angoumois Grain Moth	Red Flour Beetle	Lesser Grain Borer	Rice Weevil
365.4	1	65.5	104.7	76.9	63.9	16.8	39.2
	3	143.6	328.7	173.7	125.9	25.6	48.7
	5	212.7	279.7	163.8	85.5	27.6	40.2
	7	249.8	465.8	239.7	100.7	29.7	46.2
404.7	1	42.2	13.0	13.4	38.5	18.0	26.7
	3	93.0	140.0	122.4	89.1	20.0	37.8
	5	144.3	183.5	149.8	86.6	21.0	50.4
	7	102.4	166.1	144.8	103.9	37.0	33.7
546.1	1	117.6	68.6	122.9	74.6	15.0	33.2
	3	168.6	145.5	169.3	105.9	29.8	49.1
	5	186.9	195.8	209.1	87.5	30.6	49.1
	7	153.9	215.1	216.8	143.0	57.6	52.2

the Indian-meal moth, almond moth, Angoumois grain moth, and lesser grain borer continued to increase over the entire range of intensities tested. The response of the rice weevil and red flour beetle tended to level out or decrease when the relative intensity was increased above three times normal. A summary of the results for the night series which included intensities from one through seven times normal is shown in table 3. Again the flat grain beetle showed insufficient response to determine significant results. The results obtained for intensities from 1 through 11 times normal during day tests were similar but could not be compared on a numbers basis, since there was no means of adjusting for numbers in the test chamber for a given test.

Figure 3 shows the response of the Indian-meal moth to various intensities, indicating no change in waveband preference as the intensity was increased. This negative response was true for all insects tested except the almond moth the preference of which changed from 546.1 mμ to 365.4 mμ as the intensity was increased. Even though the spectral response does not change for most insects, the results indicate intensity is of great importance.

DISCUSSION.—The results of the spectral-response studies compare favorably with those of previous investigators for insects having negative phototropism. However, other investigators showed the peak response was at 365.4 mμ, whereas the present research showed the peak response was near 500 mμ for most species. Bertholf (1931) showed the maximum response for the honeybee to be at 365.4 mμ with a lesser peak at 553 mμ. For low intensities, Weiss' (Weiss *et al.* 1941, 1942) results showed the greatest attraction to a wavelength of 365 with the 492 mμ wavelength secondary. It can be seen that the results of Bertholf's and Weiss' researches show the same wavebands at which peak responses occur as determined in this study, except that the order is reversed. Other investigators have shown similar results. It is believed that previous investigators found the ultraviolet, 365.4 mμ, to be most attractive because mercury arc lamps having a principal output at 365.4 mμ were used as a source of ultraviolet, while tungsten lamps having a continuous spectral output were used for longer wavelengths.

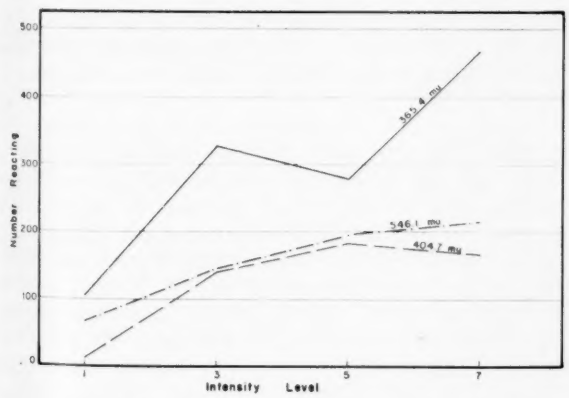

FIG. 3.—Response of the Indian-meal moth to various light intensities.

When Weiss increased the intensity by 30 times, the major peak was at 492 mμ with the 365.4 mμ waveband becoming secondary. At this intensity, the output from the tungsten lamp used as a source of 492 mμ probably became more nearly equal to the output of the mercury arc. Low intensities were used in the studies conducted at College Station. Attention is also directed to the fact that the intensity studies showed no general change in spectral response. It is also believed that the intensity measuring devices used by previous investigators did not show the true intensity. This apparent fact probably led the investigators to believe they had equal intensities when the intensity at 365.4 mμ was much higher than that at longer wavebands. The results reported herein do indicate that intensity is of utmost importance. Referring to figure 3, the unattractive waveband at 404.7 mμ became more attractive than 365.4 mμ when its intensity was increased by as much as three times that of the attractive waveband.

The results of the present research show that insects are subject to changing their preference, depending on the choice offered them. This fact explains why the yellow lamp, sometimes called an insect-repellent lamp, does not attract many insects under usual conditions. Generally, a lamp emitting more attractive wavebands (for instance

white light) will be in the vicinity, and therefore the insect is attracted to lamps other than the yellow. Referring to figure 2, it can be seen that the yellow (575 to 600 mμ) is relatively unattractive to insects when compared with 365 or 500 mμ. However, this sensitivity is near the peak of that of the human eye and therefore produces fairly desirable light for human visibility. If this same yellow light were isolated from lamps emitting more attractive wavelengths of radiation and presented to the insects alone, it would then be more attractive.

In performing this research it was noted that the relative size of the catch was closely correlated with the population existing in the test chamber at the time of the test. Apparently, therefore, a trap light could be a valuable tool in determining the degree of infestation in a grain warehouse. It must be recognized, however, that the light can attract only those insects capable of flying, so only a partial sampling of the species present may be obtained.

The results from this study indicate that a low-wattage lamp having a relatively large output in the 475 to 525 mμ wavelength region would be most efficient for trap lights. A lamp having an output of 5 to 15 watts should be adequate for use in grain warehouses. However, a test should be made under actual conditions, since the area and distance involved in a warehouse would be considerably greater than those used in the described tests.

ACKNOWLEDGMENTS.—The author wishes to acknowledge the assistance of A. W. Hartstack, Jr. in design, collection of data, and statistical analysis. Acknowledgment is also due K. H. Norris for his valuable suggestions in design and calibration of the instrumentation. The Farm Electrification Research Laboratory of the Agricultural Research Service, U. S. Department of Agriculture, located at College Station, Texas, is also acknowledged for furnishing part of the equipment and facilities used in this research.

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A Laboratory Method for Evaluating the Toxicity of Insecticides to the Garden Symphylid, *Scutigerella immaculata* (Newport)[1]

J. S. WATERHOUSE[2]

ABSTRACT

A laboratory method was developed for evaluating the toxicity of insecticides to the garden symphylid, *Scutigerella immaculata* (Newport). This method will reduce the time spent in screening insecticides in extensive field plots. With such a technique, only the more desirable materials need be taken to the field for further analysis. In the laboratory tests, parathion was more toxic to the symphylid than aldrin or malathion. These data substantiated results obtained from using the same three materials in the field.

A laboratory method was developed for evaluating the toxicity of insecticides to the garden symphylid, *Scutigerella immaculata* (Newport). Parathion, malathion, and aldrin were selected for the tests because they have been thoroughly examined under field conditions for value in garden symphylid control in Washington. Howitt & Bullock (1955), after screening 18 insecticides, found that parathion at 5 lbs. actual per acre, applied in July, gave outstanding control in large plots of cauliflower and cabnage. A direct comparison of field and laboratory results was obtained for the three materials, as well as an evaluation of the technique employed in the laboratory.

METHODS AND MATERIALS.—All feeding instars and both sexes of the symphylid were used to determine if there were any differences in susceptibility to the insecticides. Two months before the toxicity tests, many symphylids were kept in stender dishes containing moist plaster of paris (Michelbacher 1938, Waterhouse 1956). Temperature was maintained at 70° F. and relative humidity in the cultures at 100%. Each dish was replenished with a fresh lettuce leaf daily. By using the

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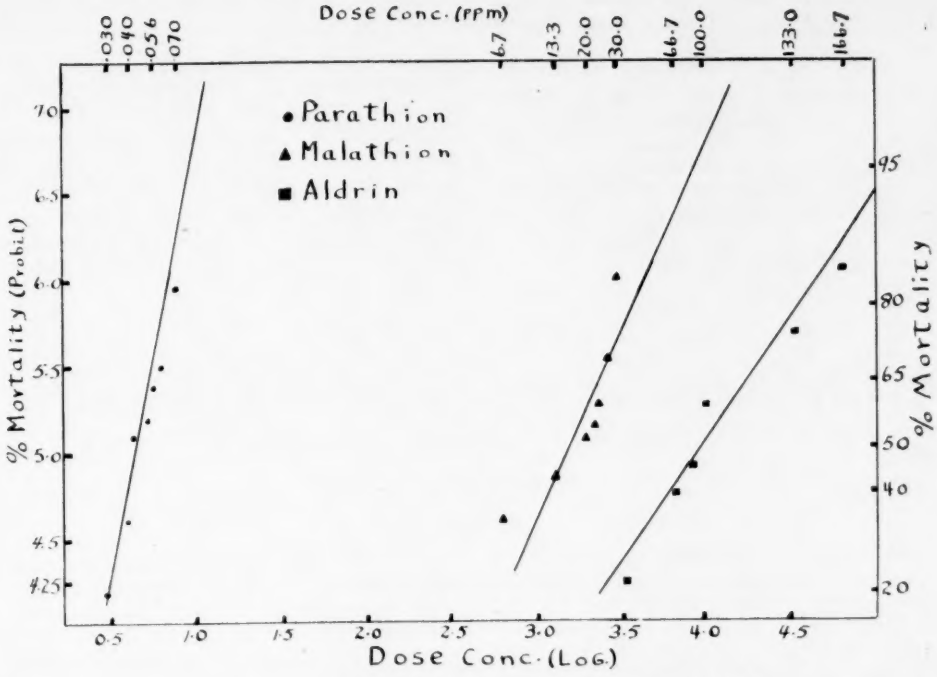

FIG. 1.—Dosage mortality comparisons of aldrin, malathion, and parathion on *Scutigerella immaculata*, each with a 24-hour exposure period.

stender dishes, the symphylids were readily available for the insecticide test. The temperature and humidity at which the dishes were maintained were optimum for symphylid maintenance.

The percentage purity for each of the three insecticides was: parathion 99, malathion 95, and aldrin 93. Each insecticide dosage, dissolved in pure acetone, was added to a given amount of sand (pH 7.0) and thoroughly mixed. The sand was then placed in a fume hood for 15 minutes and when dry was leveled over the surface of the moist plaster of Paris in a stender dish. A few drops of distilled water were added to the surface of the sand and the dish was replaced in the fume hood for an additional minute. The distilled water hardened the surface of the sand, enabling the symphylids to be kept in sight throughout a test. The moist medium beneath the previously dampened sand prevented the latter from drying. As a result, no symphylids died because of desiccation. The dishes were covered and held at the same constant temperature and humidity. At the end of 24-, 48-, and 72-hour exposures the mortality in each dish was recorded.

Neutral sand proved to be the most satisfactory test substrate. Symphylids immediately introduced to acetone-treated sand suffered high mortality in control dishes. The sand was dried in the fume hood for 15 minutes before the animals were introduced. Little or no mortality occurred within the first 24 hours but deaths increased considerably afterward because of desiccation and partial burial in the sand. Moistening the surface of the sand with a few drops of distilled water eliminated these two problems. The addition of water in some way "activated" the acetone remaining in the sand and slight mortality again occurred in the controls. However, when the dish was placed in the fume hood for 1 minute and the animals then were introduced to the still moist sand, no mortality occurred in any of the controls. The moist plaster of Paris medium beneath the sand prevented the latter from drying over the 72-hour exposure period.

Addition of a small amount of distilled water and a period of 16 minutes in the fume hood undoubtedly diluted the original insecticide dosage in the sand. Although the original dosage in each test was altered, it may be assumed each replicate was similar in that all were treated alike. For each dosage there were four replicates and a control, each containing 30 symphylids.

RESULTS.—Figures 1, 2, and 3 graphically illustrate the differences in toxicity of the three insecticides to the garden symphylid. At the end of 48- and 72-hour exposures, the curves for malathion and aldrin are almost parallel—indicating a similar degree of toxicity. The

Table 1.—LD50s and LD95s of aldrin, malathion, and parathion for the garden symphylid. Pullman, Washington. 1956.

MATERIAL (% CONCENTRATION)	EXPOSURE (HOURS)	LD50 (P.P.M.)	LD50 (LB./A.)	LD95 (P.P.M.)	LD95 (LB./A.)
Parathion	24	0.045	0.090	0.097	0.194
(0.0001)	48	0.033	0.066	0.065	0.130
	72	0.028	0.056	0.060	0.120
Malathion	24	15.8	31.6	89.1	178.2
(0.01)	48	7.1	14.2	86.7	173.4
	72	4.4	8.8	47.3	94.6
Aldrin	24	94.4	188.8	260.0	520.0
(0.1)	48	46.0	92.0	177.3	354.6
	72	27.4	54.8	135.5	271.0

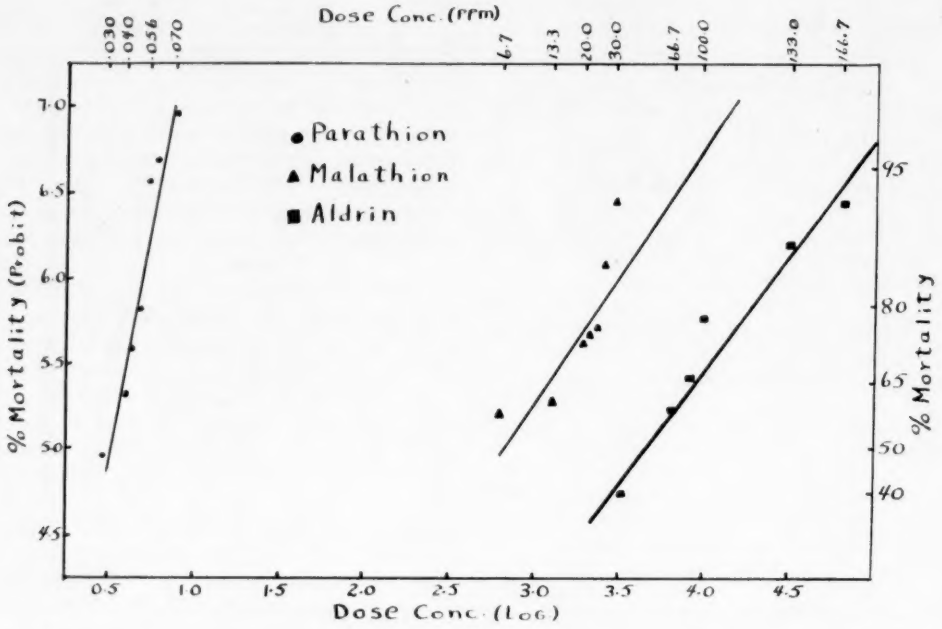

FIG. 2.—Dosage mortality comparisons of aldrin, malathion, and parathion on *Scutigerella immaculata*, each with a 48-hour exposure period.

curves for parathion at all three exposures are consistently more vertical (less range in dosage) and are widely separated from those of malathion and aldrin. These data indicate that parathion is more toxic than malathion or aldrin to the symphylid.

The LD50's and LD95's for each material are listed in table 1 as parts per million and pounds per acre. The dosage rate in pounds per acre, computed on the basis of 2 million pounds of soil to 6-inch plow depth, can be obtained by multiplying the p.p.m. by 2. The LD95's for parathion give a lower dosage than the one recommended for field control (Howitt & Bullock 1955). In the labora-

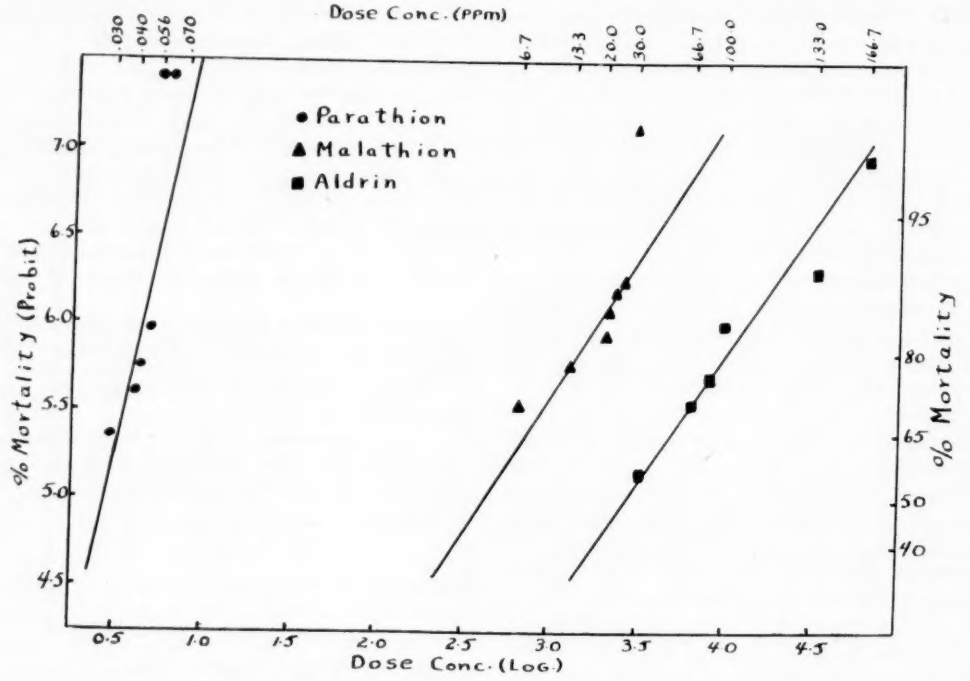

FIG. 3.—Dosage mortality comparisons of aldrin, malathion, and parathion on *Scutigerella immaculata*, each with a 72-hour exposure period.

tory test, unlike conditions in the field, the symphylid population in each test dish was always in contact with the insecticide-treated sand. In the field, only a certain percentage of the entire population is in contact with the insecticide at a given time. This variable and others occurring under field conditions account for the variance between field and laboratory results. The LD95's in pounds per acre for malathion and aldrin were very high, which fact also parallels their observed ineffectiveness in field trials (Howitt & Bullock 1955, Waterhouse 1956). No apparent differences existed between sexes and instars in susceptibility to the three materials tested in the laboratory.

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Natural Control Studies of the Melon Leaf Miner, *Liriomyza pictella* (Thomson)[1]

EARL R. OATMAN[2]

ABSTRACT

The results of 2 years' study of the natural control of the melon leaf miner, *Liriomyza pictella* (Thomson), by parasitism are discussed. The melon leaf miner is heavily parasitized in nature. Parasitism was low early in the growing season, but increased as the melon plants approached senescence, until the population level of the parasites exceeded that of the melon leaf miner. A close correlation was noted between the population numbers of the host and its parasite, except during the middle of the growinr season, at which time the population of the melon leaf mineg greatly exceeded that of its parasites. Evidently some additional limiting factors existed which were acting to reduce in number the melon leaf miner as it approached the peak of its population. The use of DDT and parathion depressed the populations of both the host and its parasites, but had less residual effect on the melon leaf miner, thus permitting it to increase in numbers more rapidly than its parasites. At least 19 species of hymenopterous parasites were reared from the larval and pupal stages. The two most numerous species were *Solenotus begini* (Ashm.) and *Halticoptera aenea* (Walk.).

Investigations of the natural control of the melon leaf miner, *Liriomyza pictella* (Thomson), were conducted during the course of studies on the life history and ecology of the species (Oatman & Michelbacher 1958, 1959).

Various species of hymenopterous parasites are known to attack related species of *Liriomyza* in Europe, South America, West Indies, and Hawaii. In the United States parasites of *Liriomyza* spp. were reported by Webster & Parks (1913), Crawford (1913), Gahan (1917), Tilden (1950), and Hills & Taylor (1951, 1953). Hills & Taylor (1951) reared 15 species of hymenopterous parasites from species of *Liriomyza* infesting lettuce and cantaloupe in Arizona. However, only Michelbacher *et al.* (1951, 1952, 1955), discussed the parasitism affecting the leaf miner now known as *L. pictella*. Field observations during 1951, 1952, and 1953 by these authors indicated that the melon leaf miner was heavily parasitized by numerous species of hymenopterous parasites, the percentage increasing as the growing season progressed.

METHODS AND MATERIALS.—A detailed study of the numbers and species of parasites was begun by the writer in July 1954 and continued through the fall of 1955. Random samples of mined melon leaves of all varieties were taken from fields in the Patterson and Vernalis area of the San Joaquin Valley of California. These were examined microscopically in the field to determine parasitism at 1- to 2-week intervals throughout the season. Random samples of leaves were also collected from check plots in two different fields of Cranshaw melons in which chemical control investigations were being conducted. These samples were collected at 1- to 2-week intervals from July 9 to October 15, taken to the laboratory in acetyl acetate bags and placed in parasite-rearing containers. The parasites were determined by B. D. Burks and C. F. W. Muesebeck of the Insect Identification and Parasite Introduction Section, U. S. Department of Agriculture. The same procedure was followed in 1955 except that the samples were taken from a field of Persian melons from July 7 to September 25, and later from a field of casaba melons from July 19 to October 3. The fields during both years were sampled from the time the plants were young, 6 to 8 inches high, until senescence. Mined leaves of other varieties of melons from different fields throughout the area were sampled at various times and handled as previously described.

RESULTS AND DISCUSSION.—The results (figs. 1 and 2) show that parasitism increased as the crops matured. A correlation of parasitism with the numbers of the melon leaf miner seemed apparent. Generally the population level of the parasites during the early stages of plant growth was below but commensurate with that of the melon leaf miner, then it lagged behind for a period, and finally surpassed that of the melon leaf miner in total numbers as the melon plants decreased in vigor. The population level of the parasites was low in comparison with that of the melon leaf miner during the early stages of growth in the Cranshaw melon field "B." As the plants

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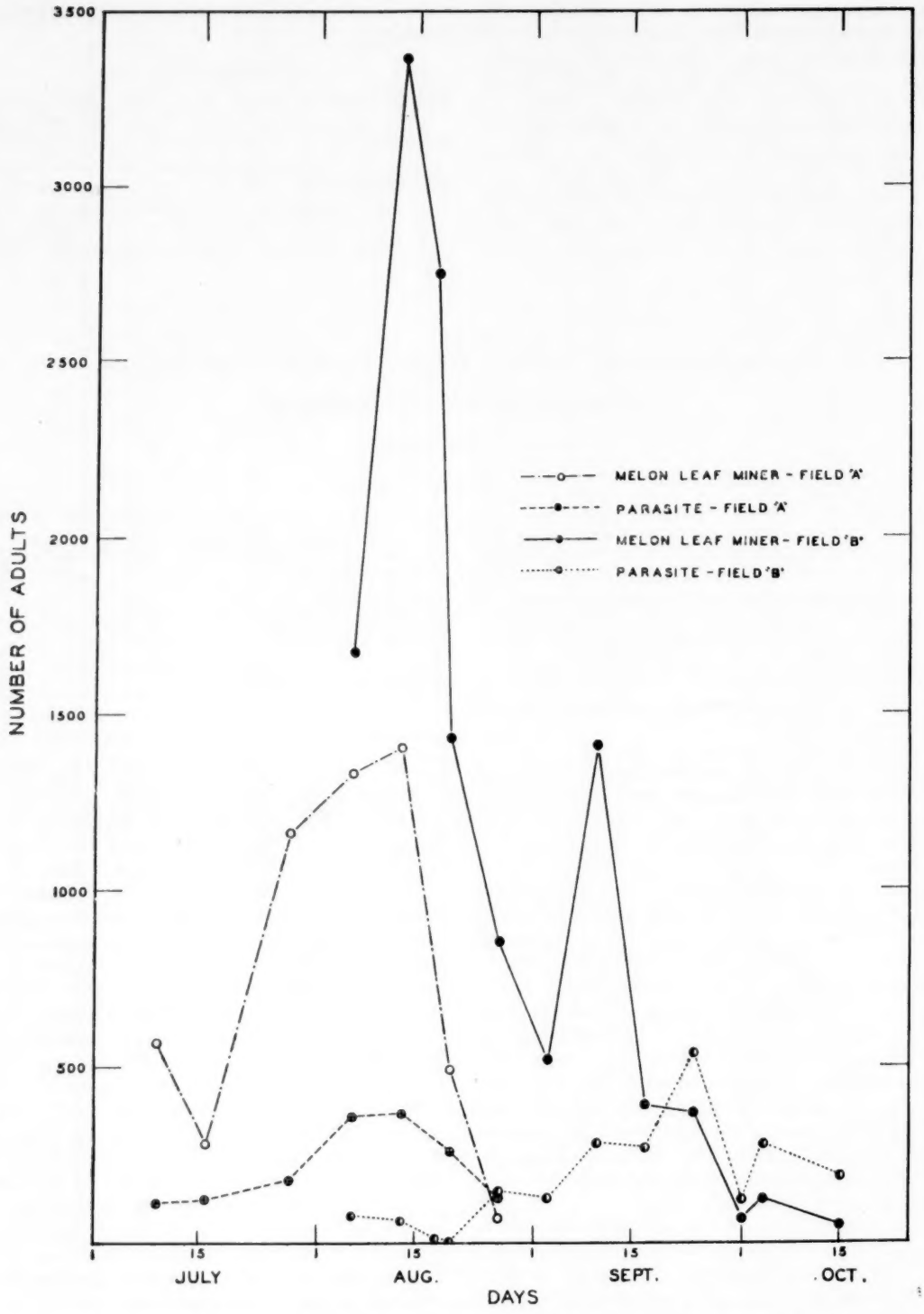

FIG. 1.—Population trend of *Liriomyza pictella* (Thomson) and its hymenopterous parasites in two Cranshaw melon fields during 1954.

approached senescence the population level of the parasites steadily increased until it surpassed that of the host. The population then fluctuated at a level with, but higher than that of the melon leaf miner.

The melons of the Persian variety sampled in 1955 were planted 2 to 3 weeks earlier than the casabas. Thus when the Persian melon field received an early treatment of DDT or parathion for insect control, some of the side effects were expressed in the depressed population levels of both the parasites and the melon leaf miners on the

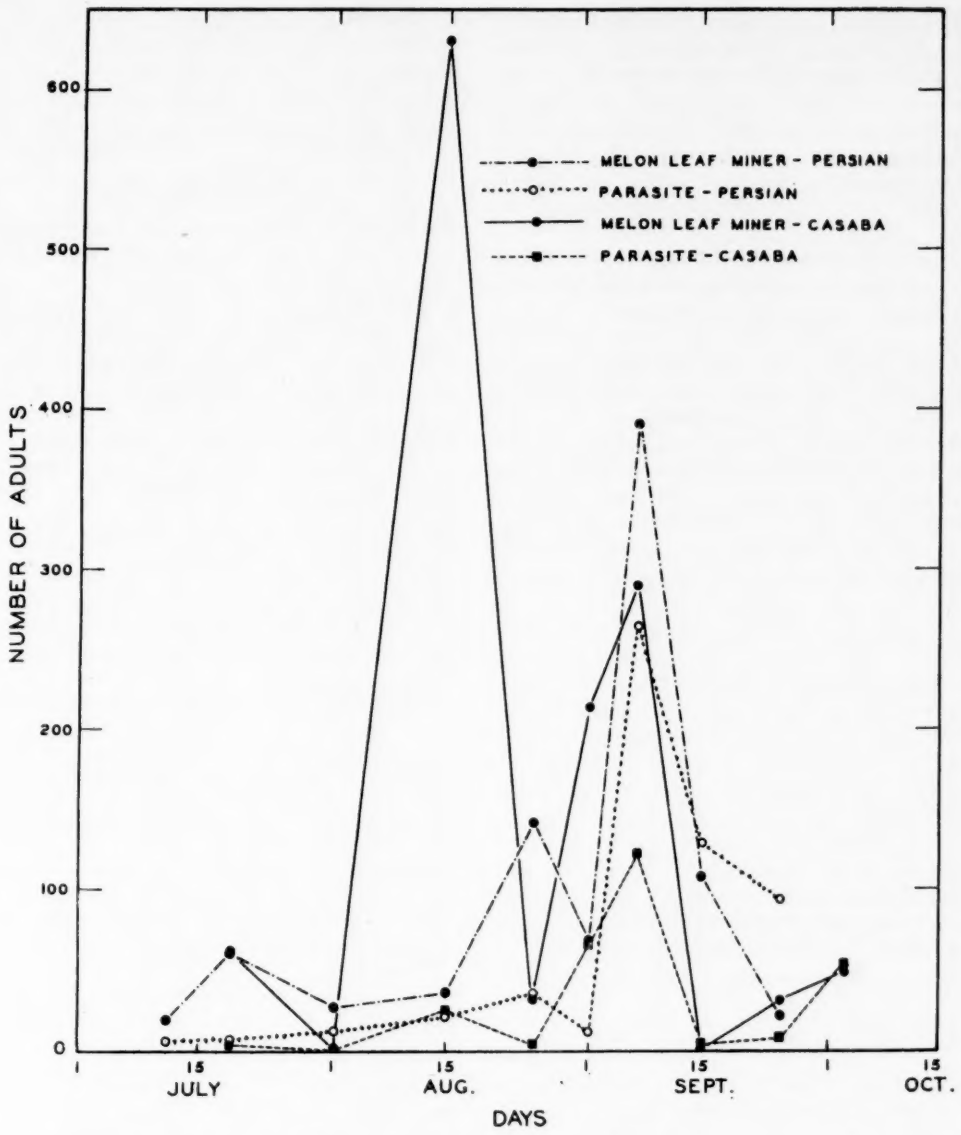

FIG. 2.—Population trend of *Liriomyza pictella* (Thomson) and its hymenopterous parasites on Persian and casaba melon plants during 1955.

nearby young casaba melon plants. The data reveal that, after such a treatment during late July, the parasites were reduced to zero and the leaf miner to only 2 per 20-leaf samples. Within the next 2 weeks the leaf miner population increased rapidly whereas the parasites increased very slowly. Hills & Taylor (1951, 1953) also noted that when DDT was applied to cantaloupe fields in Arizona the material was ineffective against *Liriomyza* but killed its parasites, allowing an increase of the leaf miner population. This phenomena has been reported by several workers for related species of *Liriomyza* from various sections of the United States.

Parathion dusts were applied by the grower to the casabas approximately August 17 and September 7. This resulted in a reduced population of both *L. pictella* and its parasites. Such treatments during the height of the growing season apparently gave impetus to the succeeding generation of both populations. However, near the end of the growing season, the melon leaf miner and its parasites were slower to recover from the insecticidal treatment and never did reach their previous numbers. The parasites, which always seemed to recover more slowly, finally exceeded the populations of the melon leaf miner toward the end of the season.

The population data obtained from the Cranshaw melon fields for both the melon leaf miner and its parasites during 1954 showed more clearly the population growth during the season than that obtained from the Persian and casaba melon fields during 1955. The latter studies, however, provided a better basis for understanding the effect of commercial insecticide treatments upon the populations.

The results of these studies show that the melon leaf miner is heavily parasitized in nature and that the widespread use of chlorinated hydrocarbons for the control of melon insect pests, has interfered with natural control by hymenopterous parasites. Parasitism is intensified and becomes more important toward the end of the season. It seems apparent that other factors are present, acting to help bring about natural reduction of the population.

A total of at least 19 species of hymenopterous parasites from 6 families was reared from the larval and pupal stages of *Liriomyza pictella* during 1954 and 1955. Most of the species were in the family Eulophidae. The two most numerous species were *Solenotus begini* (Ashm.) (Eulophidae) and *Halticoptera aenea* (Walk.) (Pteromalidae). The latter species emerges from the pupal stage, whereas the former emerges from the larval stage. These parasites were recorded by Hills & Taylor (1951) as two of the three most important species parasitizing *Liriomyza* sp. on cantaloupe and lettuce in Arizona.

The following parasites were reared from *L. pictella* during 1954 and 1955:

PTEROMALIDAE:
Halticoptera aenea (Walk.)
EULOPHIDAE:
Solenotus begini (Ashm.)
Solenotus pulchripes (Cwfd.)
Solenotus websteri (Cwfd.)
Derostenus arizonensis Cwfd.
Derostenus variipes Cwfd.
Diaulinopsis callichroma Cwfd.
Chrysocharis ainsliei Cwfd.
Chrysocharis parksi Cwfd.
Chrysocharis spp. (3 lots identified to sp. only)
Zagrammosoma mirum Grlt.
Zagrammosoma spp. (2 lots identified to sp. only)
Closterocerus trifasciatus Westwood
Astichus sp.
BRACONIDAE:
Opius suturalis Gahan
Opius spp. (3 lots identified to sp. only)
ENCYRTIDAE:
Mirini
MYMARIDAE:
Polynema sp.
SCELIONIDAE:
Telenomus spp. (2 lots identified to sp. only)

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Parasites Associated with Seed Chalcids Infesting Alfalfa, Red Clover, and Birdsfoot Trefoil Seed in New York[1]

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ABSTRACT

A series of surveys conducted in New York over a 4-year period has established that the following parasites occur in association with seed chalcids infesting alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) seed in the State: *Tetrastichus bruchophagi* Gah., *Eupelmus allynii* (Fr.), *Eupelmella vesicularis* (Ratz.), *Liodontomerus longfellowi* (Gir.), *Liodontomerus perplexus* Gah., *Amblymerus bruchophagi* Gah., and *Habrocytus medicaginis* Gah. Two of these species, *T. bruchophagi*, and *H. medicaginis*, were reared from seed chalcid infested seed of all three plant species. *A. bruchophagi* and *E. vesicularis* were reared from both alfalfa and birdsfoot trefoil seed. *L. longfellowi*, *L. perplexus*, and *E. allynii* were found to restrict their activities to seed chalcid infested seeds of a single plant host, being reared solely from seeds of red clover, alfalfa, and birdsfoot trefoil, respectively.

The name clover seed chalcid (*Bruchophagus gibbus* (Boh.)) is commonly applied to eurytomids which feed upon the seeds of alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and birdsfoot trefoil (*Lotus corniculatus* L.). However, evidence would indicate that three distinct species of seed chalcids are involved, each

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one restricted to a single genus of plants. In Russia, on the basis of biological and morphological studies, the seed chalcids associated with alfalfa, red clover, and birdsfoot trefoil have been designated as *B. roddi* Guss., *B. gibbus* (Boh.), and *B. kolobovae* Fed., respectively (Kolobova 1950, Nikolskaja 1952, Fedoseeva 1954, 1956). Here in the United States, this classification has not as yet been adopted. However, there appears to be justification for the division, in that Hansen (1955), and Neunzig & Gyrisco (1958) have demonstrated that seed chalcid populations associated with alfalfa, red clover, and birdsfoot trefoil in this country are also each restricted to species of *Medicago*, *Trifolium*, and *Lotus*, respectively.

Numerous chalcidoid parasites are associated with each of the three populations of seed chalcids. Some information pertaining to these forms has been accumulated in the past mostly incidental to studies on the seasonal history of the hosts. The most noteworthy contributions have been supplied through the efforts of Urbahns (1916, 1917, 1919, 1920). A summary of the findings of Urbahns together with data gathered by other workers in the United States has recently been brought together by Hansen (1955) and Butler & Hansen (1958). Nikolskaja (1932, 1934b) has also published several papers concerned with this group of insects in Russia; much of this information has been included in a recent work (Nikolskaja 1952) concerned with the chalcdioid fauna of the U.S.S.R.

Several of the aforementioned authors have noted that some parasites are apparently only associated with seed chalcids within the seed of a particular host plant, or with two closely related host plants. For example, *Liodontomerus longfellowi* (Gir.) has been reported as being reared only from seed chalcid-infested red clover seeds, and *Liodontomerus perplexus* Gah. is reported solely from seed chalcid infested-alfalfa and bur clover (*Medicago hispida* Gaertn.) seed.

The present paper is concerned with investigations conducted in New York, which were designed to determine which parasites of the seed chalcids occur there, thus supplementing data regarding the known distribution of these forms in the United States, and, in addition, to establish more conclusively whether certain of these parasites are host specific.

MATERIALS AND METHODS.—Samples of the seed of alfalfa, red clover, and birdsfoot trefoil were collected in New York at numerous locations in the south, central, and northern regions of the State over a 4-year period. Each sample consisted of 100 to 200 seed heads constituting several thousand seeds. The samples were placed in separate containers and the parasitic species were reared from the material and identified.[3] The majority of the samples were collected from roadside and fallow areas, with a smaller number being gathered from commercial plantings. In addition to obtaining the seed of the three plant species from various locations where more or less pure stands of these plants occurred, collections were made during the last year of the investigations at six areas where established mixed stands of all three plant species existed; it was expected that collections from the latter locations would reveal more conclusively the parasite-host relationships.

RESULTS AND DISCUSSION.—Data pertaining to the parasites obtained from seed collected at the six locations of mixed stands, and summarized data representing all samples obtained during the study, are presented in tables 1 and 2, respectively.

Two species of parasites were found to be associated with seed chalcids within the seed of all three species of plants. These were *Tetrastichus bruchophagi* Gah., and *Habrocytus medicaginis* Gah. The lack of preference found to be exhibited by these insects is in agreement with the results of investigations by Hansen (1955). In addition, if the reports of earlier workers, such as Folsom (1909), Sorenson (1934), Urbahns (1917, 1920), Crevecoeur (1946), and Nikolskaja (1932, 1934a, 1934b), are analyzed as a group they also, for the most part, substantiate the lack of host selectivity of *T. bruchophagi* and *H. medicaginis*. One exception, concerning the catholic habits of *H. medicaginis* appears in the literature; Urbahns (1920) states, "From all observations, *Habrocytus medicaginis* attacks the chalcis-fly only when the latter infests the seeds of alfalfa. . . . This parasite has never been found upon the same host within red clover seeds." However, a review of Urbahns' work reveals a greater emphasis on the study of populations of seed chalcids associated with alfalfa, and this fact therefore suggests that the apparent restriction of the parasite noted by Urbahns was possibly because of insufficient sampling of red clover plantings.

T. bruchophagi, in New York, appears to be the most numerous parasite of seed chalcids occurring in red clover and birdsfoot trefoil seed. It occurs only sporadically in association with seed chalcids within alfalfa seed. *H. medicaginis*, on the other hand, is the dominant parasite of seed chalcids within alfalfa seed, and is of lesser importance in reducing populations of seed chalcids associated with red clover or birdsfoot trefoil in the State.

Amblymerus bruchophagi Gah. was collected from both seed chalcid infested alfalfa and birdsfoot trefoil seed. This species was not found parasitizing seed chalcids in red clover.

The finding of *A. bruchophagi* in New York is believed to be the first record of the occurrence of this insect in eastern North America; also, this is the first record of its occurrence in association with seed chalcids within birdsfoot trefoil seed. Previous reports by Urbahns (1920), Sorenson (1934), Nikolskaja (1932, 1934a, 1934b and 1952), and Hansen (1955) have limited the host of this species to seed chalcids within alfalfa seed.

A. bruchophagi was found to be the second most abundant parasite within seed chalcid infested alfalfa seed and the third most abundant parasite within infested birdsfoot trefoil seed.

Two species of *Liodontomerus* were reared from a number of the samples collected; *L. longfellowi* from seed chalcid infested seeds of red clover, and *L. perplexus* from seed chalcid infested seeds of alfalfa. In the area of mixed stands of alfalfa, red clover, and birdsfoot trefoil, where seed chalcid larvae were present within the seeds of all three plant species, these two torymids exhibited conclusively a restricted host association. It is possible, at least with *L. longfellowi*, that the restriction to a particular host may be a result of the synchronization of early seasonal

[3] The authors wish to thank B. D. Burks, Entomology Research Division, Agricultural Research Service, United States Department of Agriculture, for identifying the parasitic species reported upon in this study.

Table 1.—Parasitic chalcidoids reared from samples[a] of seed chalcid infested seed of alfalfa, red clover, and birdsfoot trefoil collected from established mixed stands of the three plants at six locations in New York.

Date Sample Collected	Seed Chalcid Host	Parasitic Chalcidoids Reared from Samples						
		T. bruchophagi	*E allynii*	*E. vesicularis*	*Liodontomerus longfellowi*	*Liodontomerus perplexus*	*A. bruchophagi*	*H. medicaginis*
		Binghamton, Broome Co.						
8-7	alfalfa	0	0	0	0	2	18	45
7-19	red clover	6	0	0	0	0	0	0
8-7	birdsfoot trefoil	0	1	15	0	0	0	65
		Minetto, Oswego Co.						
8-17	alfalfa	0	0	0	0	0	0	0
8-2	red clover	1	0	0	0	0	0	0
8-8	birdsfoot trefoil	56	0	0	0	0	0	10
		Montour Falls, Schuyler Co.						
8-7	alfalfa	2	0	6	0	4	48	39
7-16	red clover	1	0	0	2	0	0	0
7-20	birdsfoot trefoil	10	17	1	0	0	88	58
		Schoharie, Schoharie Co.						
7-26	alfalfa	2	0	0	0	0	0	0
7-26	red clover	1	0	0	2	0	0	0
7-26	birdsfoot trefoil	173	0	0	0	0	0	8
		Westport, Essex Co.						
8-22	alfalfa	0	0	0	0	0	0	4
8-10	red clover	1	0	0	0	0	0	0
8-22	birdsfoot trefoil	1	0	0	0	0	4	2
		W. Jurius, Ontario Co.						
7-27	alfalfa	0	0	4	0	2	7	0
7-20	red clover	3	0	0	0	0	0	0
7-20	birdsfoot trefoil	3	0	21	0	0	28	21

[a] 200 seed heads, constituting several thousand seeds, of each plant species collected at each location.

activity of the parasite with host availability rather than a matter of host preference. Red clover fruits somewhat earlier than either alfalfa or birdsfoot trefoil, and seed chalcid infested red clover seed is prevalent at an early date. This earlier maturity of red clover is reflected in the data presented in table 1, indicating the sample collection date.

As mentioned previously, data in agreement with these findings concerning *L. longfellowi* and *L. perplexus* can be found in the literature. The former species has previously been reported solely from seed chalcid infested red clover seed by Urbahns (1920), Hansen (1955), and Butler & Hansen (1958) in the United States, and by Nikolskaja (1934a, 1952) in Russia. The latter species has been reported upon by these same workers, and with the exception of one record, all specimens of this insect have been obtained from seed chalcid infested alfalfa or bur clover. Hansen in 1955 reported that he reared six individuals of *L. perplexus* from a sample of red clover seed collected in California. However, this same author (Butler & Hansen 1958) states "This species (*L. perplexus*) is only associated with the clover seed chalcid on alfalfa." It therefore appears that the earlier statement made by Hansen is possibly in error.

Table 2.—The average and maximum percentages of occurrence of parasitic chalcidoids in association with seed chalcids within the seeds of alfalfa, red clover and birdsfoot trefoil compiled from the examination of 139[a] samples collected in pure and mixed stands of the plants in 20 counties in New York over a 4-year period.

Seed Chalcid Host		Percentage of Occurrence in Seed Chalcid Infested Seed						
		T. bruchophagi	*E. allynii*	*E. vesicularis*	*Liodontomerus longfellowi*	*Liodontomerus perplexus*	*A. bruchophagi*	*H. medicaginis*
Alfalfa	Average	2.9	0.0	1.5	0.0	3.0	5.0	15.6
	Maximum	6.5	0.0	3.6	0.0	8.0	9.4	30.0
Red clover	Average	31.2	0.0	0.0	27.9	0.0	0.0	6.1
	Maximum	81.4	0.0	0.0	51.9	0.0	0.0	10.5
Birdsfoot trefoil	Average	12.8	0.9	0.8	0.0	0.0	4.5	5.6
	Maximum	45.9	2.6	2.3	0.0	0.0	18.2	18.0

[a] 25 samples of alfalfa, 53 samples of red clover, and 61 samples of birdsfoot trefoil. Each sample consisted of 100 to 200 seed heads, constituting several thousand seeds.

In regard to the abundance of *L. longfellowi* and *L. perplexus* in New York, *L. longfellowi* was the second most abundant parasite reared from samples of red clover, and *L. perplexus* emerged from, on the average, 3% of the alfalfa seeds infested with seed chalcids.

Two species of the family Eupelmidae, *Eupelmella vesicularis* (Retz.), and *Eupelmus allynii* (Fr.), were reared in relatively small numbers from the seed samples —*E. vesicularis* from infested alfalfa and birdsfoot trefoil seed, and *E. allynii* from infested birdsfoot trefoil seed. The occurrence of *E. vesicularis* in association with the seed chalcid in birdsfoot trefoil seed has not previously been reported; Sorenson (1934) has reported this parasite only from samples of seed chalcid infested alfalfa seed. *E. allynii* has previously not been reported as being associated with seed chalcid infested seed. Both *E. vesicularis* and *E. allynii* were always collected from seed samples yielding numerous other parasites. These circumstances and the general knowledge that many eupelmids have the habit of developing as hyperparasites (Clausen 1940), suggest that, at least in some instances, these forms may be parasitizing chalcidoids other than seed chalcids within the legume seeds.

A summary of the known host relationships of the parasites associated with seed chalcids infesting alfalfa, red clover, and birdsfoot trefoil seed based on the above findings in New York, together with information compiled from the literature, is presented in table 3.

Table 3.—A summary of the known host relationships of parasitic chalcidoids reared from seed chalcid infested seed of alfalfa, red clover, and birdsfoot trefoil compiled from the literature[a] and including the findings of the present study.

Chalcidoids	Source of Chalcidoids as Designated by Authority Reporting Relationship (H.—Hansen; Ni.—Nikolskaja; N. & G.—Neunzig & Gyrisco)		
	Infested Seed		
	Alfalfa	Red Clover	Birdsfoot Trefoil
Tetrastichus bruchophagi	H., Ni., N. & G.	H., N. & G.	H., N. & G.
Eupelmus allynii			N. & G.
Eupelmus atropupureus		Ni.[b]	
Eupelmus microzonus		Ni.[b]	
Eupelmella vesicularis	N. & G.	Ni.[b]	N. & G.
Liodontomerus insuetus	H.	Ni.	H.
Liodontomerus longfellowi		H., Ni., N. & G.	
Liodontomerus perplexus	H., Ni., N. & G.	[c]	
Amblymerus bruchophagi	H., Ni., N. & G.		N. & G.
Habrocytus medicaginis	H., Ni., N. & G.	H., N. & G.	H., N. & G.
Trimeromicrus maculatus	H.		H.

[a] Includes Hansen (1955), and Nikolskaja (1952).

[b] Nikolskaja in 1932 and 1934 publications included these species in association with seed chalcids in alfalfa seed.

[c] Hansen in 1955 stated that he reared *L. perplexus* from seed chalcid infested red clover seed. However, in a recent paper (Butler & Hansen 1958) the following statement is made: "This species (*L. perplexus*) is only associated with the clover seed chalcid on alfalfa."

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Experimental Prevention of Bark Beetle Transmission of *Ceratocystis ulmi* (Buis.) Moreau with the Systemic Insecticide Chipman R-6199[1]

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Abstract

Trunk-implantation treatment of American elm trees with any of three dosages of the systemic insecticide Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate [hydrogen oxalate salt]) was effective in preventing bark beetle transmission of *Ceratocystis ulmi* (Buis.) Moreau. Successful bark beetle transmission of the fungus in control trees occurred only from beetle cagings during the interval from May 20 through June 10. During this period, 30% of the control trees became infected with the fungus and showed symptoms of the disease. The mean length of beetle-feeding niches was always 1.5 mm. or less in treated trees, and 3.3 mm. or greater in control trees. In every diseased tree, the minimum-length niche that penetrated at least to the cambium was 3 mm. long. On the basis of this limited knowledge of niche-length requirements, a 96.3% reduction in the number of infectible niches resulted from insecticidal treatment. The insecticide, at 20 grams per tree, was effective in reducing the length of feeding niches for the entire 80-day study period following treatment.

Dutch elm disease, caused by *Ceratocystis ulmi* (Buis.) Moreau, has been the most serious shade-tree disease in the United States since 1930, when the causal fungus was first discovered in this Country. The present control of this plant pathogen is attempted through (1) sanitation, which involves destruction of all known elm wood infected with the fungus or infested with the insect vectors; and (2) insecticidal spraying of elms and elm wood to control the common fungus vectors, *Scolytus multistriatus* (Marsh.) and *Hylurgopinus rufipes* (Eich.). Intensive practice of both control methods is necessary if the disease is to be controlled.

Insecticidal control methods, utilizing DDT as a dormant or foliar spray, were first developed by Whitten & Parker (1945). These control methods have been effective in supplementing sanitation measures where the disease occurs. However, such controls have limitations and disadvantages that make them undesirable. The thorough coverage of the tree bark is frequently not accomplished because of unfavorable spraying conditions and careless sprayer operation. The spray must be applied while the trees are dormant if adequate coverage is to be obtained. For the protection of associated wildlife and plants, the approved spray period in some areas is further limited. In addition, tree owners voice objection to messy spray residues on objects under or adjacent to sprayed trees. Because of these related problems, the possible use of systemic insecticides applied to the soil and tree bark or implanted in the tree trunk has been investigated. Systemic types of insecticidal treatments could lessen many of the present causes for objections to efforts to control elm bark beetles and could possibly yield more effective control.

Previous work with systemics in controlling tree insects has been noted by Al-Azawi *et al.* (1958). These workers also reported preliminary studies on the effectiveness of several systemic insecticides in the control of *S. multistriatus*.

During 1958, the effectiveness of several systemic insecticides in preventing the bark beetle transmission of *C. ulmi* was studied under field conditions. The methods, results, and discussion of findings with the systemic Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate [hydrogen oxalate salt]), are presented in this paper.

Methods.—During 1958, studies were conducted on American elm trees, *Ulmus americana* Linnaeus, located in forest conditions in Milwaukee County, Wisconsin. Trees of uniform size, 2-inch D.B.H. and 20 feet tall, were used. The trees were located in an area where the disease was not prevalent so experimental conditions were uniform throughout the area. Trees, numbering 220, randomly divided into 2 equal blocks, were involved in the experiments. One block of 110 was further divided into 2 equal groups, 55 being treated with the Chipman R-6199, and the other 55 left as a control on insecticidal treatment. The other block of 110 was left as controls on natural spread of the fungus.

In the insecticide-treated trees, one of three dosages of the technical grade insecticide, 10, 20, or 30 grams per tree, was applied; one-fourth of the total dosage was placed in each of four holes using a 50% aqueous solution of the insecticide in a pipette. The number of treated trees and treatment date with each respective dosage were as follows: 10 grams, 10 trees, May 2; 20 grams, 20 trees, May 13; and 30 grams, 25 trees, May 12. Four ½-inch holes, bored at right angles to each other around the base of the trunk approximately 6 inches from soil level, served as implantation sites. The exact height of holes was staggered slightly to minimize any injurious effect of the borings upon the tree. The holes were slanted downward at a 45° angle to the main axis of the tree and were plugged with corks after application of the insecticide.

Beginning May 20, and continuing at approximately weekly intervals through August 1, 10 newly emerged *S. multistriatus* beetles, that had been placed on laboratory cultures of *C. ulmi* for 24 hours, were enclosed in a sleeve-type cage on one random terminal branch of each test tree. The cages enclosed approximately a 3-foot section of the branch. Five insecticide-treated trees and five control trees were exposed to fungus-bearing beetles on each caging date. All five caged insecticide-treated trees for a given date received the same dosage of insecticide. The beetles were left on the tree for 1 week after which beetle mortality and feeding data were taken from the en-

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Table 1.—Number of American elm trees per 5-tree plot infected with *Ceratocystis ulmi* by allowing 10 *Scolytus multistriatus* beetles bearing the fungus to feed on each individual tree for 1 week.

Treatment	Caging Dates										
	5/20	5/29	6/4	6/10	6/16	6/21	6/27	7/11	7/18	7/25	8/1
R-6199[a]	0	0	0	0	0	0	0	0	0	0	0
Control	2	2	1	1	0	0	0	0	0	0	0

[a] Tree dosage in plots of June 4 and 27 was 10 grams; in plots of June 10 and 16, July 25, and August 1, 20 grams; and in plots of May 20 and 29, June 21 and July 11 and 18, 30 grams.

closed section of each test branch. The number and length of feeding niches were recorded.

Subsequently, each tree was observed weekly for symptoms of the Dutch elm disease. As soon as any questionable symptoms were detected in test trees, twig samples were taken from the affected portion of the tree, and laboratory cultures of the elm tissue were made on a sterile medium composed of ground elm tissue, potato extract, 2% dextrose, and agar. Only trees yielding positive cultures of *C. ulmi* were considered infected with the disease.

RESULTS AND DISCUSSION.—Treatment of the experimental trees with 10, 20, and 30 grams of Chipman R-6199 was effective in preventing bark beetle transmission of *C. ulmi* (table 1). Successful transmission of the fungus in control trees occurred only in the first four weekly cagings on May 20 and 29, and June 4 and 10 (table 1). Six of the 20 control trees caged during this period died of the disease. Successful transmission took place at the same time as vigorous spring growth in elms.

The mean number, mean number to the cambium, and mean length of beetle-feeding niches per tree for the various caging dates are presented in table 2. The mean values are based on 5 trees and 10 beetles per tree per caging date. In considering the data in table 2, the most significant difference between treated and untreated control trees is in the length of the feeding niches. To the present time our research has indicated that niches 3 mm. long or longer are generally required for successful transmission of the fungus and infection of the tree through terminal twigs. The number of feeding niches is apparently not important as long as the length is less than 3 mm. This apparent niche-length requirement is further supported by the length of feeding niches that occurred in caged branches of the trees that died of the disease in this study. In every diseased tree, the minimum-length niche that penetrated at least to the cambium was 3 mm.

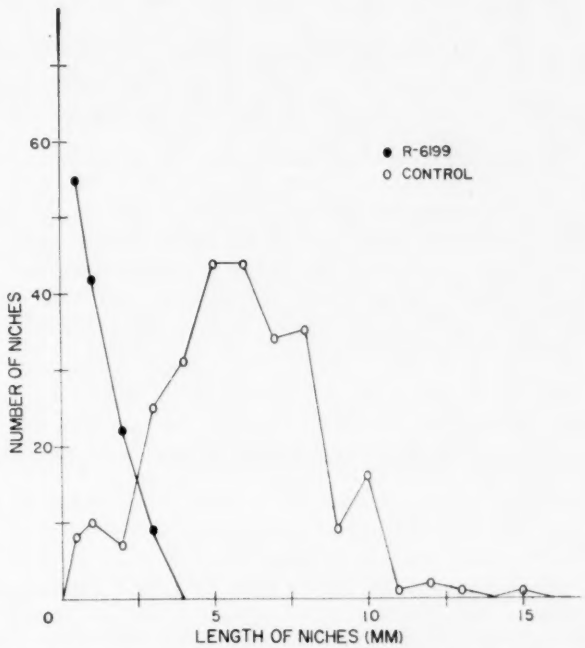

FIG. 1.—Number of beetle-feeding niches in 55 R-6199 treated and 55 control trees, graphed according to their length.

In table 2, the mean length of niches per tree for each caging date was 1.5 mm. or less in treated trees and 3.3 mm. or greater in control trees. However, 9 of 127 niches in treated trees were 3 mm. long (figure 1). Though these nine niches could probably have served as suitable inoculation sites, no disease occurred. In figure 1, the niches occurring in 55 insecticide-treated and 55 control trees are

Table 2.—Feeding data for 10 *Scolytus multistriatus* beetles caged on each tree of 5-tree plots for a 1-week period.

Treatment	Caging Dates											Total Mean per Tree
	5/20	5/29	6/4	6/10	6/16	6/21	6/27	7/11	7/18	7/25	8/1	
Mean No. niches per tree												
R-6199	4.2	2.6	3.6	1.0	1.6	3.6	1.2	2.4	2.6	2.2	0.4	2.4
Control	7.8	3.0	5.2	3.8	3.6	8.4	4.0	6.6	2.2	6.6	2.8	4.9
Mean No. niches to cambium per tree												
R-6199	1.8	1.6	1.4	0.0	0.6	1.4	0.6	0.4	0.4	0.6	0.2	0.8
Control	7.4	3.0	4.8	3.0	3.6	8.4	3.8	6.6	1.8	6.6	2.6	4.7
Mean length of niches (mm.)												
R-6199	0.9	1.5	1.2	0.7	1.1	1.3	0.9	0.6	0.7	1.3	0.8	1.0
Control	5.8	4.9	6.4	3.7	4.9	6.4	6.8	5.7	3.3	6.4	5.6	5.7

Table 3.—Residual effectiveness of Chipman R-6199 in controlling twig feeding by *Scolytus multistriatus*.

Dosage of R-6199 per Tree (Grams)	Days from Treatment to Caging	Mean No. Niches to Cambium/Tree[a]	Mean Length of Niches/ Tree (Mm.)
10	33	1.4	1.2
10	56	0.6	0.9
20	28	0.0	0.7
20	34	0.6	1.1
20	73	0.6	1.3
20	80	0.2	0.8
30	8	1.8	0.9
30	17	1.6	1.5
30	40	1.4	1.3
30	60	0.4	0.6
30	67	0.4	0.7
Control	—	4.7[b]	5.7

[a] Based on 5 trees and 10 beetles per tree.
[b] Based on 55 trees and 10 beetles per tree.

graphed according to their length. The occurrence of only 127 niches in treated trees versus 269 in control trees represents a 52.7% reduction in the number of niches owing to insecticidal treatment. Furthermore, only 7.1% of the niches in insecticide-treated trees, as compared with 90.0% of the niches in control trees, were apparently long enough to serve as inoculation sites for the fungus. Thus, 96.3% reduction resulted in suitable niches.

At 20 grams per tree, R-6199 was effective in reducing the number and length of beetle-feeding niches throughout the experimental period of 80 days. Tree dosages of 10 and 30 grams were respectively effective in assays 56 and 67 days after treatment. The full length of residual effectiveness of these treatments was not determined. However, in other beetle assays of trees 1 year after treatment with R-6199, effective control of beetle feeding has been obtained. The data in table 3 indicate that treatment of trees with R-6199 in May will provide protection against beetle transmission of the fungus throughout the important period of fungus transmission in spring and early summer.

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Biology of *Philaenus leucophthalmus* (L.), in Massachusetts[1]

Robert Lavigne[2,3]

Abstract

Knowledge concerning the biology of the meadow spittlebug, *Philaenus leucophthalmus* (L.), is expanded to cover Massachusetts conditions. Surveys showed that this pest is present throughout the State; seven of the eight North American varieties are recorded according to their abundance. Seventeen previously unreported host plants are listed. Prediction of egg hatching apparently is feasible; hatching began when the number of accumulated day degrees above a base of 50° F. dated from April 1 reached 75 ± 3 degrees. Data are included which validate Weaver & Whitney's sampling technique utilizing the percentage of infested stems as an index to the abundance of nymphs.

Comparatively little is known about the meadow spittlebug, *Philaenus leucophthalmus* (L.), as a pest of forage crops in Massachusetts. C. H. Fernald (1891) presented a brief outline of the seasonal history of this insect within the State. Preliminary biological observations were reported by Shaw *et al.* (1953) in a report on forage crop insects for 1952. The work summarized in the present paper was undertaken to provide more information on this pest under Massachusetts conditions.

Methods.—Weekly surveys were conducted in six fields over a 2-year period, with the exception of December through March. Three of the fields were practically pure stands of alfalfa, one was a mixture of alfalfa and grass, one a stand of red clover, and the last a mixed clover stand. Fifty sweeps with a 12″ collecting net were taken in each field at each sampling. Rearing studies and observations on the mating habits of the adults were carried out in the laboratory.

Results.—Statewide surveys have shown the meadow spittlebug to be present throughout Massachusetts. Seven of the eight North American varieties listed by Doering (1930) occur here; only the variety *lateralis* has not been collected thus far. Varietal distribution occurs as follows: *pallidus*, 34.8%; *spumarius*, 56.4%; *fabricii*, 3.1%; *leucophthalmus*, 1.2%; *marginellus*, 2.5%; *leucocephalus*, 0.5%, and *fasciatus*, 1.5%.

Host Plants.—Note was taken of 17 previously unreported host plants of the nymph of *P. leucophthalmus*. The common names are taken from Gray's Manual of Botany, 8th Edition and are here listed:

Botanical Name	Common Name
Ambrosia trifidia L.	great ragweed
Aster cordifolium L.	heart shaped aster
Aster ericoides L.	white heath aster
Bromus secalinus L.	chest or chess grass
Cirsium vulgare (Savi) Tenore.	bull or common thistle
Galium asprellus Michx.	rough bedstraw
Lactuca scariola L.	prickly lettuce, compass plant
Oxalis stricta L.	sheep sorrel
Physalis sp.	Chinese lantern
Potentilla norvegica L.	rough cinquefoil

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Potentilla recta L.	common cinquefoil
Rhus typhina L.	staghorn sumac
Rudbeckia hirta L.	yellow daisy, black-eyed susan
Saponaria vulgaris L.	bouncing bet
Solidago juncea Ait.	goldenrod
Spiraea tomentose L.	hardhack, steeple-bush
Ulmus rubra Muhl	slippery elm

Biology.—Philaenus leucophthalmus overwinters in the egg stage in Massachusetts. Mild temperatures in late April and early May induce hatching. After eclosion the nymph crawls to a suitable host plant and commences feeding. Spittle is formed within a few minutes. The nymph remains within its spittle mass throughout its developmental period of 5 to 7 weeks, except during an occasional foray to a new feeding site. Nymphs become desiccated and die if deprived of spittle for more than an hour at ordinary spring temperatures.

Just prior to the final molt to the adult stage, the nymphs cease the production of spittle. The foam is allowed to dry, thus forming a chamber in which the final molt invariably occurs. The callow adult, white at first, remains within the chamber until its wings dry and its color pattern begins to take form. The adults, which emerge in great numbers in early to late June, typically show little desire to migrate until the host plants are mowed or decline in succulence. They then move on to hedgerows and surrounding fields where new growth is available and remain widely dispersed throughout the summer. In the fall the adults, mostly females, migrate back to alfalfa and small grain fields. Oviposition commences in early September, the eggs being laid in packets containing 2 to 12 eggs held together by a frothy cement. Egg packets are commonly laid between the leaf sheath and stem of the plant at a 45° angle to the axis of the stem. Apparently the seasonal history of this insect in Massachusetts is essentially the same as that reported for the Midwest.

Both Medler (1955) and Weaver (1956), using Wisconsin and Ohio data, devised methods of predicting the time of egg hatching based on the number of day degrees above a given base. Medler analyzed 6 years of spittlebug hatching data at two Ohio stations and found that by beginning on February 1, and summing day degrees above a base of 40° F., the date of hatching could be predicted within a week, the day degree total being 150. Weaver, utilizing a 50° F. base, considered an accumulation of 25 day degrees from April 1 as the optimum time to spray for spittlebugs. The present author has compared both methods under Massachusetts conditions. The 40° F. base, dating from February 1, proposed by Medler does not give consistent results under our conditions. However, the 50° F. base method dating from April 1 as proposed by Weaver has, over a 3-year period, corresponded to the date of observed hatching when the total accumulated day degrees equaled 75 ± 3.

Around Amherst first instar nymphs seem to prefer young goldenrod and bladder campion plants. In the spring these plants produce a tightly encompassed upward growth that provides the high humidity and protection from the sun that are favorable for survival of the nymphs. Both the aforementioned species are good sources of newly hatched nymphs at the beginning of hatching and might serve as indicator plants in checking infestations.

Table 1.—Percentage of meadow spittlebug nymphs of various instars present in the field at a given time during the spring of 1956.

	Instar Number					
Date	1st	2nd	3rd	4th	5th	Adult
May 17	100					
June 7	6	32	62			
June 14		7	24	30	40	1
June 22			17	34	49	Few
June 30					100	Many

Movement of a nymph is largely confined to a single host plant. Heavy rainstorms may wash nymphs from their hosts, but more often only the spittle is washed away. The position attained by the nymph places it in apposition to the most succulent part of the plant. The apparent gregariousness shown by the nymphs on a single host plant probably results from the efforts of al those present to find the most succulent part of the plant —the growing tip.

Periodical field observations were made to determine the percentage of the total population that each instar constituted in the field at any one time. These data are presented in table 1.

Laboratory rearing studies were carried on to determine the number of instars. First instar nymphs were recovered from the field and placed on young plants of bladder campion, *Silene latifolia* Brit. & Rend. Daily inspections were made of each plant and the nymphal exuviae, if any, were removed and placed in alcohol. Records made of the length of the various instars derived from the above method led to the conclusion that there were five instars.

The occurrence of five instars was further demonstrated by the application of Dyar's law (Comstock 1947) which states that a linear measurement in successive instars of larvae is in geometric progression. Thus it is possible to calculate the number of instars when the head widths of two consecutive larvae and those of the first and last instar are known. While previous workers have measured the head capsules of fresh or preserved nymphs, the present author utilized exuviae. Measurements were taken from 210 exuviae collected from both field and laboratory reared material. The average ratio between any two consecutive measurements of the series shown in table 2 was 0.69. This figure was obtained by dividing the highest measurement into that of the next earlier one,

Table 2.—Comparison of actual mean width of 210 head capsules of nymphal exuviae of the meadow spittlebug with expected widths based on Dyar's law.

Mean Widths (Mm.)	Instar Number				
	1st	2nd	3rd	4th	5th
Actual	0.41	0.61	0.95	1.36	1.77
Ratio of adjacent instars	0.67	0.64	0.69	0.76	
Expected[a]	0.40	0.58	0.84	1.22	1.77

[a] Calculated from Dyar's law.

Table 3.—Relative abundance of males and females of the meadow spittlebug in actual numbers collected by field sweeping in 1956 and 1957 at Amherst, Mass.

Year and Sex	Month				
	June	July	August	September	October
1956					
♂	192	83	206	54	13
♀	120	55	114	124	43
1957					
♂	954	79	175	15	3
♀	719	63	238	43	26

summing their ratios and obtaining the mean. A fairly constant ratio seems to exist between instars. Calculations based on the measurements obtained show that the correct number of instars is five. Actual measurements obtained show close agreement to those of Weaver & King (1954).

Adult populations were followed throughout the summer and fall of 1956 and 1957. Peak emergence was delayed a week to 10 days in fields that were mowed early and the crop left lying in the field to dry. Here the nymphs merely migrated to the stubble to complete their development. Sharp declines in adult populations occurred as a result of mowing or decrease in plant succulence. In these situations adults migrated to surrounding fields or hedgerows where succulent foliage was available. In our test fields consistently smaller populations of spittlebugs occurred in clover as compared with alfalfa. First-year alfalfa stands had heavier populations than second- or third-year fields.

Mating occurred indiscriminately both among individuals and varieties. The majority of the mating pairs found were composed of the varieties *spumarius* and *pallidus* in one combination or another. However one mating pair involved a male *spumarius* and a female *marginellus*. This mating is of particular interest because no males of the variety *marginellus* have been reported. The observations support the theory that these color varieties are not separate species. The copulatory position was maintained up to 8 hours. Remating between the same individuals was observed on two occasions.

As the season progressed the ratio of males to females in the fields under observation changed. According to the data presented in table 3, the shift occurred in late August and early September. A large part of the mating has then taken place and, since delayed fertilization takes place in this species, the females are free to oviposit when conditions are favorable. Heavy fall reinfestations of alfalfa and grain fields were largely composed of females that were seeking favorable egg deposition sites. Males, which are not driven by the same physiological requirement, remain widely dispersed.

Preliminary observations indicated that cutting a crop just prior to the period of heavy egg deposition discouraged females from using a field as an oviposition site. A quantitative study, which tended to support these observations, was made on adult spittlebug populations in two alfalfa fields under different cutting systems. (See graph 1.) Thus if a system of management is followed which involves cutting during the last week of August or first week of September, spittlebug infestations the following spring may be substantially reduced.

Table 4.—Comparison of field data with statistical data of Weaver & King (1954) for predicting spring nymphal infestation of the meadow spittlebug based on adult populations in the fall.

Field Location	Adults per Sweep in Fall	No. of Nymphs per Stem Expected in Spring[a]	Actual No. of Nymphs per Stem
Amherst	0.52	0.52	0.42
Hadley	.04	.04	.08
Southwick	.08	.08	.29
Southampton	.20	.20	.20
Westfield	.10	.10	.51
Westfield	.46	.46	.40

[a] Based on Weaver & King (1954).

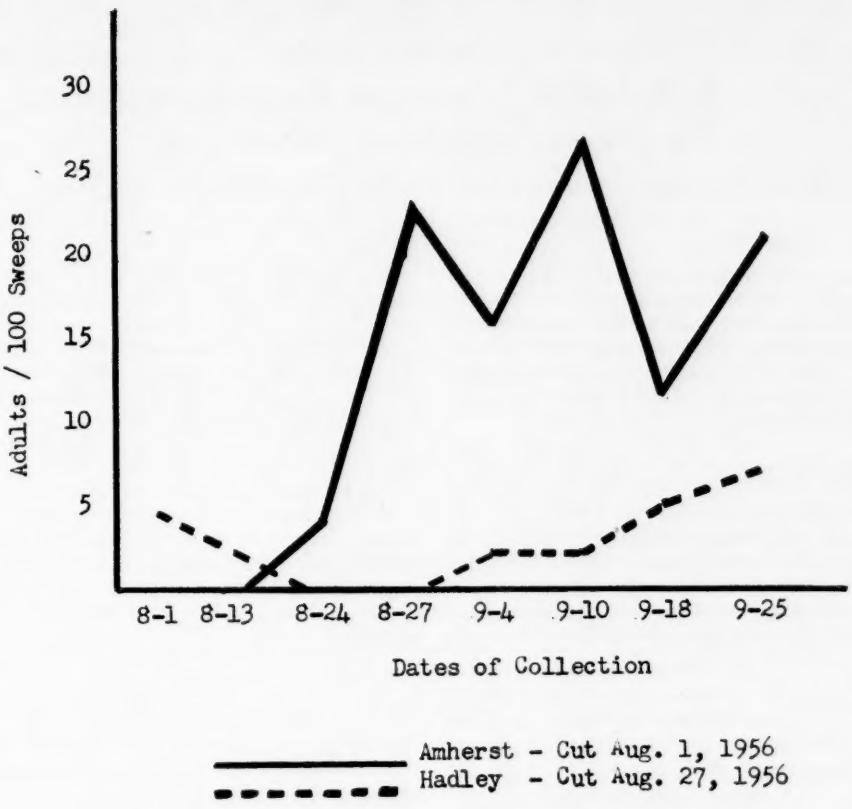
Weaver & King (1954) devised a method of predicting the nymphal infestation in the spring based on the number of adults per sweep recovered in a given field the preceding September. This method was also found to be reliable under Massachusetts conditions. Using this technique, a survey of six fields conducted in September 1956 indicated that none of them would have an economic infestation the following spring. This premise was borne out when 100 stem counts were made of these same fields in the spring of 1957, as can be seen in table 4.

Several methods for determining nymphal spittlebug abundance were evaluated. The sampling technique proposed by Weaver & Whitney (1956), which utilizes the percentage of infested stems in a given area, produced the quickest and most accurate results. The accompanying data (table 5) indicate that this statistical technique of estimating nymphs per stem from the proportion of in-

Table 5.—Field data[a] compared with statistical data of Weaver and Whitney on alfalfa for determining the reliability of using infested stems as an index to the number of nymphs of the meadow spittlebug per stem under Massachusetts conditions.

Field Location	Number of		No. of Nymphs per Stem[b]
	Infested Stems	Nymphs per Stem	
Amherst	0.25	0.42	0.45
	.22	.35	.37
	.30	.50	.55
Hadley	.10	.08	.14
	.16	.22	.25
	.16	.19	.25
Southwick	.26	.50	.46
	.09	.09	.13
Southwick Country Club	.20	.29	.33
Southampton	27	.40	.48
Westfield	.17	.20	.27
	.29	.51	.52

[a] Based on 100-stem samples.
[b] Predicted by Weaver & Whitney (1956).



GRAPH 1.—Comparison of effect of early and late cutting of alfalfa stands on adult meadow spittlebug populations in 1956.

fested stems is applicable under Massachusetts conditions.

The economic importance of the meadow spittlebug as a pest of forage crops was investigated. Population studies indicated that approximately 17% of the alfalfa fields surveyed in 1957 could be regarded as sufficiently infested to warrant treatment if an average of ½ nymph per stem is considered the criterion of economic importance.

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Effectiveness of New Insecticides on the Potato Leafhopper and the Influence of Leafhopper Control and Potato Variety on Tuberworm Infestations[1]

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ABSTRACT

In 11 experiments insecticides were screened as foliage sprays for insecticidal activity against the potato leafhopper, *Empoasca fabae* (Harr.), on Irish potatoes. Four weekly applications of Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), Sevin® (1-naphthyl-*N*-methyl carbamate), and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) at dosages as low as 0.2 pound per acre were still highly effective 7 days after the last treatment, showing a 98% reduction in leafhoppers at that time. Ethion, Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) gave good results at 0.5 pound actual per acre. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dibrom® (*O,O*-dimethyl-*O*-(1,2,-dibromo-2,2-dichloroethyl phosphate), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), parathion, and Phosdrin® (1-methyloxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) applied at 0.5 pound actual per acre produced a high initial mortality but afforded only a limited residual action. DDT at 0.5 pound actual was quite effective but was inferior to Sevin, Thiodan, and a DDT-Dibrom combination. Aldrin, dieldrin, and heptachlor did not give adequate leafhopper control.

Observations in commercial plantings showed that damage by the potato tuberworm, *Gnorimoschema operculella* (Zell.), to tubers in the soil was directly influenced by the degree of potato leafhopper control and the potato variety. Where poor leafhopper control was obtained 63% of the Cobbler tubers were infested with tuberworms as compared with an infestation of only 1.5% in the Pungo variety. Tuberworm damage to Cobbler tubers was very light in fields receiving adequate leafhopper protection.

Normally the potato leafhopper, *Empoasca fabae* (Harr.), causes comparatively little damage to the spring Irish potato crop on the Eastern Shore of Virginia. In 1957, however, a very severe leafhopper outbreak developed which reduced potato yields as much as 50%. The magnitude of the leafhopper infestation was influenced by three factors: 1) Much of the damage occurred during the 4 weeks immediately preceding harvest, at which time the regular insect control programs had been largely discontinued because of the very light potato flea beetle (*Epitrix cucumeris* (Harr.)) infestation; 2) rainfall was extremely light all spring and the potato plants were in poor condition to withstand leafhopper attacks; and 3) many of the growers had shifted to dieldrin as *the* potato insecticide, because of the resistance of the potato flea beetle and Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) to DDT. Although dieldrin had suppressed the relatively light leafhopper infestations in the past, it failed almost completely during the 1957 outbreak.

The investigations reported in this paper were undertaken as part of an extensive program to find a better all-around potato insecticide.

MATERIALS AND PROCEDURE.—Eleven experiments involving 98 different treatments were conducted at Painter, in Accomack County, Virginia, in 1957 and 1958. All insecticides were tested as sprays in the emulsion form except Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) (soluble powder) and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine 3-methyl) phosphorodithioate) (wettable powder). The test materials were applied in 100 gallons of water per acre by means of a knapsack sprayer operating at 40 pounds pressure. The potato plots (variety Irish Cobbler) consisted of five rows, each 30 feet long with three untreated rows between each plot. All treatments were replicated four times in randomized blocks.

The relative effectiveness of the various insecticides was evaluated by counting the numbers of leafhopper nymphs on 25 different potato leaves selected at random from the middle row of each plot.

In addition to the screening trials, field observations were made on the general effectiveness of commercial spraying practices for leafhopper control. High pressure sprayers were used in applying emulsifiable concentrates of DDT, dieldrin or parathion to adjacent plantings of the Pungo and Cobbler varieties. The relationship between these two varieties and their response to different degrees of leafhopper control were noted. Plant condition was used as a basis for evaluating the several treatments.

RESULTS AND DISCUSSION.—Four weekly applications of Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), Sevin® (1-naphthyl-*N*-methyl carbamate), and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*, 6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) gave excellent leafhopper control 7 days after the last treatment at dosages as low as 0.2 pound per acre (experiment 1, table 1). Dieldrin was relatively ineffective with only a 45.2% leafhopper reduction. This failure to control leafhoppers was reflected in a highly significant decrease in yield as compared with all insecticidal treatments, except Delnav at the 0.2-pound-per-acre level. The reason for the poor yield in the plots treated with Delnav was not determined.

As indicated in experiment 2 (table 1), ethion, Guthion,

[1] Presented at the 30th Annual Meeting of the Eastern Branch of the Entomological Society of America held in Baltimore, Maryland, November 24–25, 1958. Contribution from the Entomology Department, Virginia Truck Experiment Station, Paper No. 130, Journal Series. Approved for publication March 23, 1959. Accepted for publication March 30, 1959.

Table 1.—Effectiveness of insecticides as foliage sprays against the potato leafhopper on Irish potatoes. Painter, Virginia, 1957 and 1958.

Treatment and Pounds of Actual Toxicant per Acre	Potato Leafhopper Nymphs per 25-Leaf Samples on Days Shown After Last Application			
	2	7		
	Number	Number	Per Cent Reduction	Yield[a]
Experiment 1 (Spray Applications on June 4, 11, 18, and 25) 1957				
Delnav, 0.2	1.3	1.5	98.7	72.1
Delnav, 0.5	0.3	0.5	99.5	102.8
Dieldrin, 0.2	38.3	61.3	45.2	75.0
Sevin, 0.2	1.5	0.8	99.3	93.2
Sevin, 0.5	0.5	0.3	99.8	101.0
Sevin, 1.0	0.3	0.0	100.0	97.6
Thiodan, 0.2	1.5	0.8	99.3	98.3
Thiodan, 0.5	0.5	0.0	100.0	106.5
Untreated	68.5	111.8	—	71.6
L.S.D. at 5% level	19.6	13.8		14.0
at 1% level	26.5	18.7		19.0
Experiment 2 (Spray Applications on June 4, 11, 18, and 25) 1957				
Dibrom, 0.5	0.3	31.8	73.3	
Dylox, 0.5	0.8	46.5	60.2	
Ethion, 0.2	0.8	11.0	90.8	
Ethion, 0.5	0.5	12.0	90.0	
Guthion, 0.5	0.0	11.5	90.3	
Sevin, 0.5	0.0	3.8	96.2	
Trithion, 0.5	0.3	5.9	95.0	
Untreated	52.5	119.0	—	
L.S.D. at 5% level	3.1	20.4		
at 1% level	4.3	27.9		
Experiment 3 (Spray Applications June 25) 1957				
Aldrin, 0.5	36.3	102.8	56.7	
DDT, 0.5	1.0	22.3	90.6	
Delnav, 0.5	0.3	7.8	96.3	
Ethion, 0.5	16.3	26.3	88.9	
Dieldrin, 0.2	39.0	136.3	42.6	
Endrin, 0.2	11.0	23.2	90.2	
Heptachlor, 0.5	32.0	97.0	59.2	
Sevin, 0.5	0.3	23.8	90.0	
Sevin, 1.0	0.0	15.0	92.7	
Thiodan, 0.5	0.5	11.0	95.4	
Untreated	70.3	237.5	—	
L.S.D. at 5% level	7.1	22.0		
at 1% level	9.5	29.5		
Experiment 4 (Spray Applications June 4, 10, 16, and 23) 1958				
DDT, 0.5	0.8	20.5	84.2	111.8
DDT, 0.5-Dibrom, 0.5	0.0	3.8	97.1	139.5
Sevin, 0.5	0.0	4.0	96.9	127.0
Sevin, 1.0	0.3	1.3	99.0	140.3
Thiodan, 0.5	0.5	2.5	98.1	132.5
Thiodan, 0.75	0.5	1.8	98.6	145.8
Untreated	55.3	129.5	—	90.5
L.S.D. at 5% level	9.5	17.6		19.0
at 1% level	13.5	24.5		26.0

[a] Pounds of U. S. No. 1 potatoes.

Sevin, and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) continued to hold leafhopper populations in check for 1 week after the completion of a regular control schedule and were superior to Dibrom® (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate) and Dipterex in this respect. Ditman & Wiley (1958) in Maryland, obtained good residual action with Thiodan and Trithion at 0.5 pound per acre and Sevin at 1 pound per acre in tests to control the potato leafhopper on snap beans.

In experiment 3, (table 1), single applications of aldrin, dieldrin, and heptachlor were ineffective at both 2 and 7 days after application, whereas DDT, Delnav, Sevin, and Thiodan gave good initial leafhopper control and extended protection. Ethion and endrin, although showing significantly higher 2-day counts than DDT, Delnav, Sevin, and Thiodan, held the number of leafhoppers at a low level thereafter.

All treatments in experiment 4 (table 1) were superior to the untreated check. However, DDT gave somewhat poorer leafhopper control than the other insecticides as well as a definite reduction in yield. When DDT was combined with Dibrom, both leafhopper control and yield were increased to a point where they were equal to Sevin and Thiodan.

In addition to the insecticides included in the preceding four experiments, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), malathion, parathion, and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl-dimethyl phosphate, 60% technical) gave an initial leafhopper kill of 95% or higher but were characterized by a lack of residual action.

A study of the spraying practices in four commercial potato fields in Northampton County revealed a very interesting relationship between leafhopper control, potato variety and potato tuberworm, *Gnorimoschema operculella* (Zell.), infestation in unharvested tubers (table 2).

A single application of dieldrin in field 1 and two applications of dieldrin in field 2 resulted in very poor leafhopper control. Moreover, plants of the Cobbler variety were completely dead in both fields whereas plants of the Pungo variety had green main stems and a few green leaves. Although there were no apparent significant differences in the number of tuberworms in the stems of the two varieties, the unharvested Cobbler tubers had tuberworm infestations of 75% and 51% in fields 1 and 2, respectively, as compared with a 2% and 1% infestation in the Pungo tubers. This difference in tuber infestation was obviously caused by the fact that the plants of the Cobbler variety were killed prematurely by the leafhoppers, forcing the tuberworms to seek food elsewhere, whereas the plants of the Pungo variety were still relatively green and could support a heavy tuberworm population.

In fields 3 and 4, where dieldrin-parathion and DDT-parathion combinations resulted in effective leafhopper control and the plants remained green, tuberworm damage in the Cobbler variety was reduced to practically zero. It should be pointed out that tuberworm feeding patterns varied from the usual (Poos & Peters 1927, Hofmaster 1949) in that only an occasional mine was observed in the foliage.

A more extensive survey of the potato area in Northampton County showed that approximately 300 acres of potatoes were not harvested because of excessive tuberworm damage to the tubers. The heaviest infestations were always associated with the Cobbler variety and with poor leafhopper control.

In a direct comparison of the Pungo, Cobbler, and Kennebec varieties, plants of the Kennebec variety were

Table 2.—Influence of potato leafhopper control and Irish potato variety on potato tuberworm damage to potatoes in the soil. Capeville and Cheriton, Virginia, 1957.

Field Number	Insecticide, Actual Toxicant per Acre, and No. of Applications	Variety	Effect of Hopperburn on Plants June 28	No. of[a] Tuberworms per 50 Plants	Unharvested Tubers Infested with Tuberworms (%)
1	Dieldrin, 0.2 lb., 1	Cobbler	Completely dead	310	75
		Pungo	Main stalk green, a few green leaves	249	2
2	Dieldrin, 0.5 lb., 2	Cobbler	Completely dead	384	51
		Pungo	Main stalk green, a few green leaves	439	1
3	Dieldrin, 0.2 lb.+parathion, 0.25 lb., 3	Cobbler	Green stems and foliage	207	3
		Pungo	Green stems and foliage	305	0
4	DDT, 0.5 lb.+parathion, 0.25 lb., 2	Cobbler	Green stems and foliage	79	0
		Pungo	Green stems and foliage	53	0

[a] Based on plant stems only. Since most of the tuberworms had left the Cobbler vines in fields 1 and 2 it was necessary to estimate the original infestation in the Cobblers from the number of tunnels and emergence holes.

found to withstand poor leafhopper control even better than plants of the Pungo variety and to have worm-free tubers as well.

Potato varietal studies by Parker *et al.* (1954) at Onley, Accomack County, Virginia, rather clearly indicate that the difference in varietal response to leafhopper populations was due to a varietal characteristic and not influenced by spray practices. In these trials at Onley, which received no insecticidal treatments, the relative varietal response to leafhoppers was the same as that observed in the present study at Painter.

Based on the results of the screening tests and observations throughout the potato growing areas of southeastern Virginia, it has become quite apparent that aldrin, dieldrin, and heptachlor are not adequate all-around potato insecticides. However, a combination of these materials with DDT, Diazinon, or parathion has given good control of the potato insect complex in general and is being used on a commercial basis in this area. Sevin and Thiodan, since they control not only the potato leafhopper but other potato pests as well, hold considerable promise for use as a general potato insecticide.

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Field Evaluation of Demeton in the Control of Beet Yellows Virus[1]

E. S. Sylvester, Vernon E. Burton, and R. B. Duncan[2]

Abstract

Differences in yield and/or per cent sugar were found to be associated with the reduction in numbers of green peach aphids (*Myzus persicae* (Sulz.)) and delayed incidence of infection of the beet yellows virus in demeton spray-treated plots in three locations in Kern County, California. Populations of aphids were initiated by alatae early in February and were terminated by natural factors during mid-May. Symptoms of virus yellows infection were first found in early April, and spread continued until approximately 100% of the plants were infected. The best available evidence indicated that treatment could delay 100% infection for a period of approximately 3 weeks. With the exception of one field in which generally poor growing conditions prevailed, increased tonnage of beets was found in the sprayed plots. Increase in sugar percentage was associated with treatment in all locations.

Increasing concern by growers over reduced yields and sugar percentage in the beet crops in Kern County, California, necessitated some attempt at remedial measures. In the fall and winter of 1957, it was suggested that a beet-free period be instituted,[3] and within the limits of practicality the sugar-beet-growing areas of Kern County were free of crop beets for approximately 1 month from December 15, 1957, until January 15, 1958, when the first seedlings of the 1958 crop began to emerge.

In addition to the beet-free period, replicated plots were established during the spring of 1958 in three commercial beet fields in an attempt to evaluate the efficacy of vector control in reducing virus spread. One field

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[3] Because of the results of English and Continental European work and those of the U. S. Department of Agriculture workers upon the general problem of beet yellows virus, the suggestion was made and accepted by those concerned at a general meeting during October 1957 at Bakersfield, California, attended by representatives of growers, processors, the U.S.D.A., and the Experiment Station and Agricultural Extension staffs of the University of California.

(Rockwell) was north of Wasco, another (Camp and Lackenmaier) was south of Shafter, and the third (Henson Bros.) was southwest of Bakersfield in the Maricopa Flats area.[4]

METHODS.—Each experimental area consisted of four replications of a treatment and a check plot. The treated plot within the block was selected at random. Each individual plot consisted of eight rows of 30-inch beets, and although treatment was continued on the eight rows for the entire length of the field, for purposes of insect and disease counting, each plot was limited to 200 feet of length, beginning at a distance 50 feet from the edge of the field. Only the center four rows were used for sampling purposes, the two outside rows on each side serving as buffer rows.

The experiments were not designed to evaluate the efficiency of various aphicides, but rather to determine if repeated weekly applications of a known potent aphicide of the systemic type would be associated with the prevention or delay of the spread of beet yellows virus within the treated areas. Demeton[5] was chosen because it was known to be an effective aphicide and somewhat systemic in its action. It was a good compromise in the attempt to minimize the action of the insecticide on the normal populations of parasites and predators, and was considered to have a high potential for registration approval for use on sugar beets.

Insect samples were taken at weekly intervals beginning February 11 and were continued through May 13, 1958. For sampling purposes, each of the four 200-foot count rows was subdivided into ten 20-foot lengths. At random, 10 of the possible 40 lengths were selected each week for actual sampling. The individual samples were as follows: prethinned beets, 10 plants within each of the 10 selected 20-foot row lengths; thinned beets, during the month of March, 4 plants from each selected row length, after which 1 plant within each of the 10 sections selected.

The plants were left in place, examined in their entirety, and the total aphids on each plant were counted. For purposes of recording, the aphids were grouped as to the green peach aphids, *Myzus persicae*, and others. Within each of the two groups, the individuals were classified as alatae, apterae, and nymphs. While population levels remained low, records were kept as to the proportion of the nymphal population, which was alatoid, but later it was merely noted whether alatoid nymphs were present in a particular colony. The incidence of fungus disease of the aphids was noted, and records were kept of certain predators and parasites.

The presence of the following diseases was recorded: beet yellows, beet mosaic, beet yellow-net, beet curly top, and the occasional case of mildew. The most reliable estimate of the amount of disease present was made by examination of a minimum of 100 and a maximum of approximately 300 contiguous plants in one randomly selected row out of the four possible in each of the plots.

Spraying was done with a tractor-mounted, power take-off Hypro spray pump with a four-row boom. The nozzles per row progressed from one to three as the size of the beets increased, and the amount of demeton was progressively increased from 2 to 8 ounces in 10 to 40 gallons of water. Spraying was done as nearly as possible at weekly intervals beginning February 21 and ending May 6. However an unusual period of rainy weather necessitated adjustments in both the intended spraying and sampling schedules, especially the former. Only one sample was missed entirely on two of the fields, and on the third it was delayed a day on one occasion.

The treatment of the plots was continued through the entire length of the fields. Thus, to estimate yields, it was possible to subsample each plot through the length of the field. Five 20-foot sections were chosen as subsampling areas, each area including the eight plots of the four blocks. Yields were measured by weighing all beets in each of the four count rows of each plot, the beets from two rows being pooled to give a single weight value. Thus 2 weighings were made from each of the 5 subplots in each plot, or a total of 10 weighings for each of the 8 plots in each of the 3 locations. Prior to weighing, the beets were machine lifted, topped by hand, and four to six beets were bagged for sugar analysis. Weighing was done using a bucket and a spring tension scale, with recordings being made to the nearest 0.5 pound. The raw data included the weight of the bucket and the bag, and this correction factor was subtracted at the time of analysis. After the field weighing, the bagged subsamples were taken to the Sprekels Sugar Company laboratory in Bakersfield, California, where the beets were brushed, trimmed, and analyzed for sugar content. By this method, two additional figures were obtained, the per cent clean beets and the per cent sugar. The data analysis used the figures corresponding to the pounds of clean beets and those estimating the per cent sugar.

RESULTS.—*Aphid Population Development.*—*Myzus persicae* made up approximately 95% of the total number of aphids counted (something in excess of 87,000 individuals), and the conclusions drawn presumably apply to this species. The green peach aphid is also believed to be responsible for most of the field spread of beet yellows virus. This would be particularly true in the spring in Kern County, for not only are the other potential vectors, *Aphis fabae* Scop., and *Macrosiphum solanifolii* (Ashm.), less efficient in the transmission of this virus, but they are not the dominant species on sugar beets during this time.

The results of the aphid counts are given in figures 1, 2, and 3, based upon the total aphids counted, but segregated as to alatae (fig. 1), apterae (fig. 2), and nymphs (fig. 3). Each field and each sampling period was analyzed separately, assuming a Poisson distribution and using the t test of significance. In the figures, whenever two points are given for the aphid count on a particular date, the evidence for separation has statistical support at a minimum of the usual 5% level of significance.

An occasional alate was found in two of the fields during the first sampling period (February 11), and in the following weeks nymphs were found in all fields. The general population trend was similar in all of the fields, *viz.*, initiation, peaking, and termination. The rate of increase was least in the Henson Bros. field, and the population

[4] The authors wish to acknowledge the splendid cooperation of the various growers, not only for the use of the land and crop, but for their willingness to adjust their agricultural practices to fit the spraying, sampling, and harvesting schedules.

[5] The authors are indebted to the Chemagro Corporation for supplying the demeton, to the California Spray-Chemical Corporation for contributing the spray equipment, and to the Davies Machinery Company for furnishing the tractor.

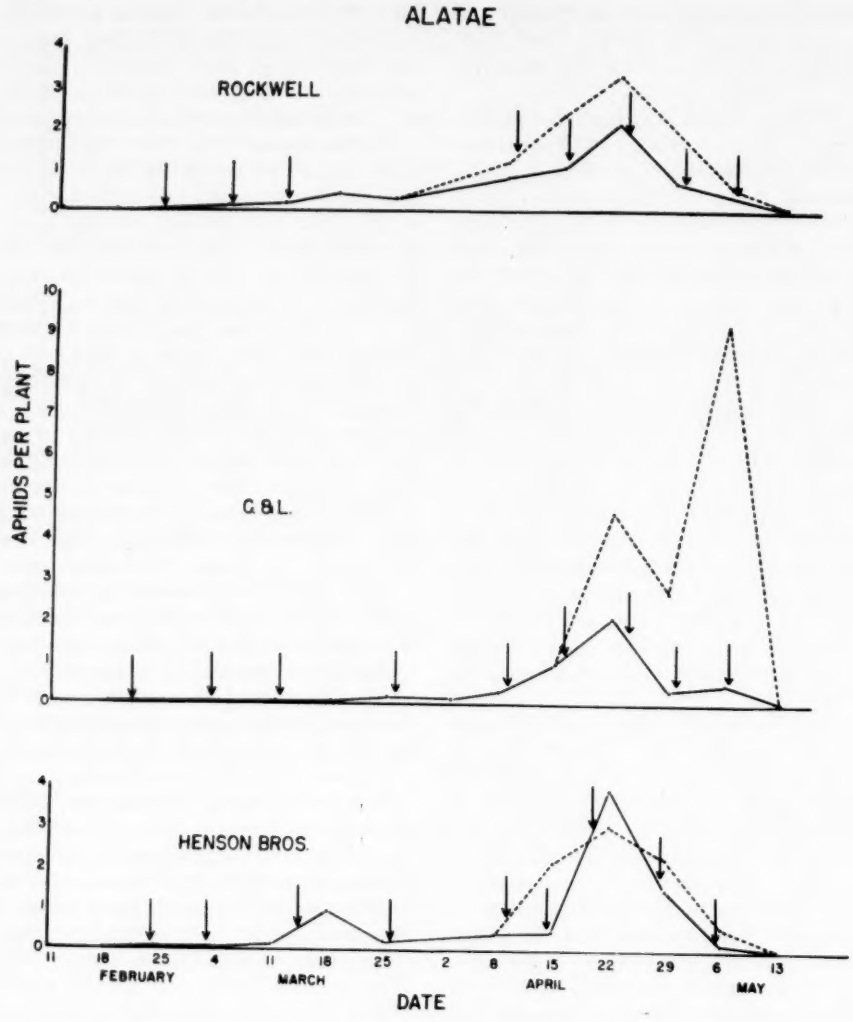

FIG. 1.—Population polygons indicating the incidence of alate aphids (mainly *Myzus persicae* (Sulz.) on demeton-treated (solid line) and untreated (broken line) plots in three beet fields in Kern County, California, during the spring of 1958. The vertical arrows indicate the dates of demeton application.

reached maximum levels a week earlier in the Rockwell field than in the other two. In all fields the population abruptly decreased during the 2-week period April 29 to May 13. Presumably host plant maturation and the onset of high temperatures typical of the summer season of Kern County, established the general limit to the length of time that the population would endure. Within this limit, other factors affected the quantitative aspects of the developmental trends in each field. With the exception of the immigrating alate aphids, it is obvious that treatment affected the numbers of insects found, but it did not affect the general developmental trends. In the case of the Rockwell field and the Camp and Lackenmaier field, treatment delayed the downward trend of the population approximately a week. There was little evidence that demeton acted in a truly systemic manner. Evidently translocation of the toxicant was limited to the leaves actually contacted with the material, and thus the new growth was continuously susceptible to colonization.

Not apparent from the counts is the fact that the population on the treated plants was limited almost entirely to the newly emerging leaves in the center of the plants, while that of the untreated plants had a greater tendency for dispersal over more of the plant tissue. However, independent of treatment, there was a general tendency for the aphids to continually relocate on the more favorable immature tissues and to avoid the mature leaves. Later in the sampling period occasional small colonies composed of a few individuals would be found on the older leaves which were approaching senescence. On the untreated plants rather considerable numbers of aphids would be found on the peripheral tissues at the base of what otherwise might be considered mature leaves. This peripheral tissue is the last to mature on the leaves. The application of demeton largely prevented this condition, with the exception of the occasional plant which apparently was missed during the treatment. The general impression was that treatment, while not suppressing

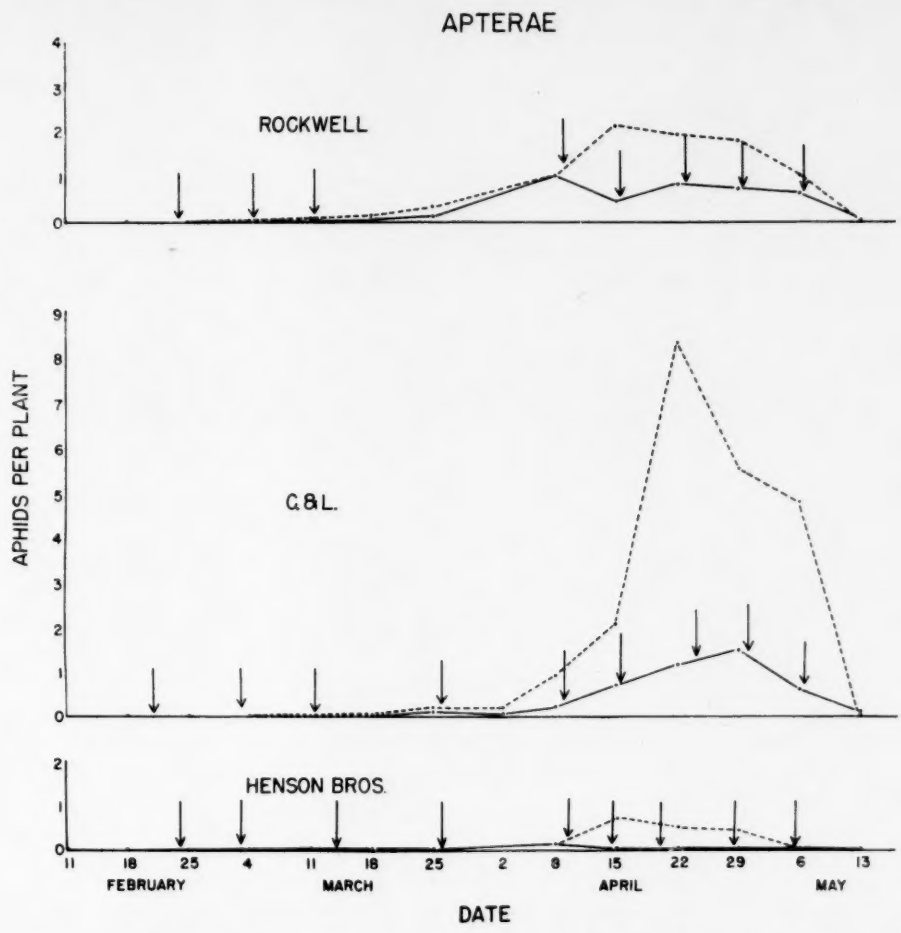

FIG. 2.—Population polygons indicating the incidence of apterous aphids on demeton-treated (solid line) and untreated (broken line) plots in three beet fields in Kern County, California, during the spring of 1958. The vertical arrows indicate the dates of demeton application.

population development completely, did, by confining it to the young center leaves of the plants, make it a more sedentary one and thereby lessened the population's effectiveness as far as virus spread was concerned.

Rockwell Field.—This field initially had the most rapidly developing population of the three sampled, and the population reached maximum approximately 1 week before either the Camp and Lackenmaier field, or the Henson Bros. field. Rain, combined with heavy soils in this location, prevented the application of demeton for approximately 1 month (April). During this period the aphid population on both the treated and untreated plots increased to an estimated level of 100% infestation, with 30 to 40 aphids per plant. Observational data indicated that although alatoid nymphal production (table 1) was initiated relatively early (March 18), and peaked about a month later when 95% of the colonies examined had alatoid nymphs among their numbers,[6] the accompanying increase in the amount of fungus disease prevented realization of the alate potential. All available evidence (table 2) indicated that the incidence of fungus attack prevented maximization of the population above an average of approximately 100 aphids per plant, pre-

Table 1.—Production of alatoid nymphs in populations of *Myzus persicae* developing on sugar beets during the spring of 1958, Kern County, California.

	Per Cent of Plants Sampled with								
	Plants[a]	Aphids	Alatoid	Plants	Aphids	Alatoid	Plants	Aphids	Alatoid
Date	Rockwell			Camp and Lackenmaier			Henson Bros.		
2/11	400	1	0	400	0	0	400	1	0
2/18	400	2	0	400	2	0	400	1	0
2/25	400	6	0	400	8	0	160	4	0
3/4	160	49	0	400	9	0	160	18	0
3/11	160	63	0	160	26	0	160	16	0
3/18	160	90	1	160	65	0	160	22	0
3/25	160	99	3	160	90	0	160	39	0
4/2	—	—	—	160	80	1	—	—	—
4/8	88	100	6	160	98	10	70	70	0
4/15	40	100	95	40	100	68	40	100	3
4/22	40	100	63	40	100	78	39	100	3
4/29	40	100	70	40	100	25	40	99	10
5/5	40	100	38	40	100	60	40	38	0
5/13	40	56	0	39	57	3	40	5	0

[a] Number of plants sampled.

[6] A subsample of aphids from plants in an untreated area taken on March 11, and examined microscopically in the laboratory, indicated that while alatoid nymphs amounted to only 2.8% of the total of 1,186 nymphs counted, they made up 30% of the population of 3rd to 4th instar nymphs.

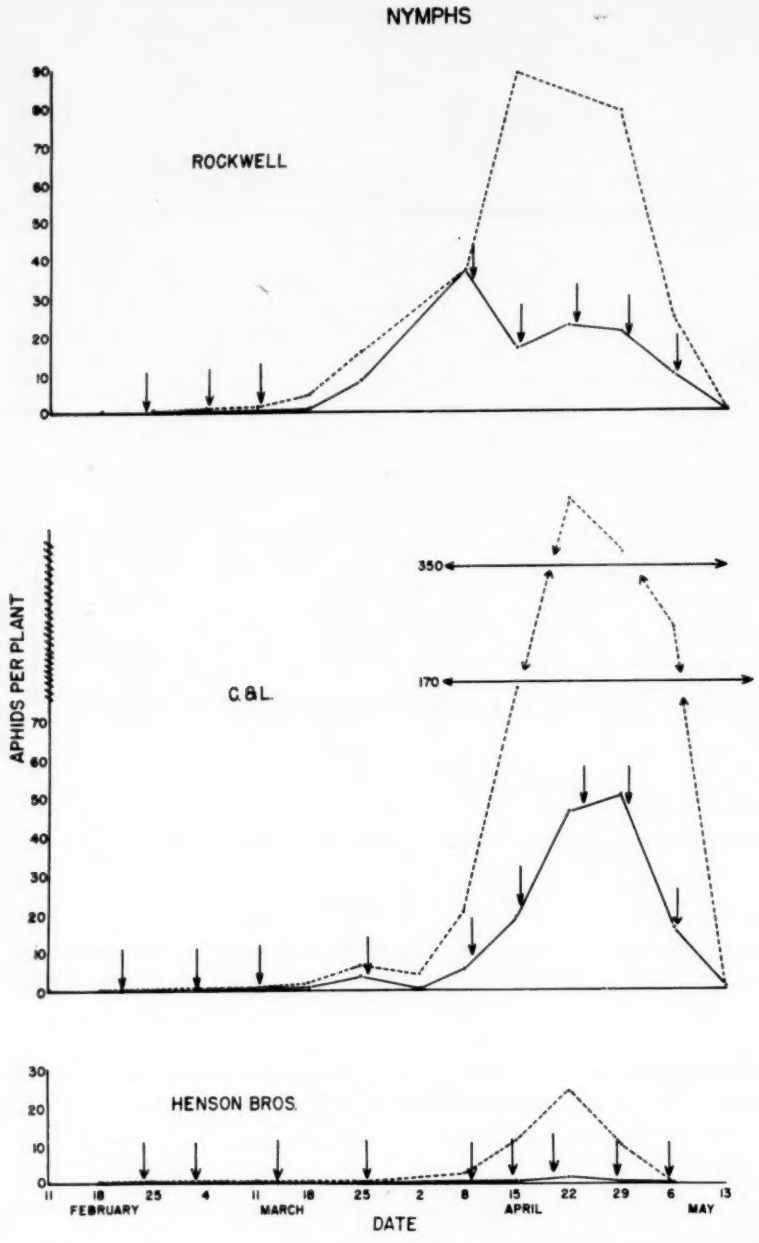

FIG. 3.—Population polygons indicating the incidence of nymphal aphids on demeton-treated (solid line) and untreated (broken line) plots in three beet fields in Kern County, California, during the spring of 1958. The vertical arrows indicate the dates of demeton application.

vented the within-field release of secondary alatae, and advanced the date of population decline (figures 1 and 3). Coccinellids appeared relatively late, but were judged to have been in sufficient numbers to insure cleanup of the residual population after May 13 (table 2). Braconid parasites were probably of little consequence in this field.

Camp and Lackenmaier Field.—The population in this field peaked about a week after that in the Rockwell field, and simultaneously with that in the Henson Bros. field. Initial alate immigration was lower than in either of the other two fields, but the population maximized at the highest level found, an average of approximately 370 nymphs per plant on the untreated plots. This field had a lighter soil and slightly less rainfall compared with the Rockwell field, and the fungus disease was slower in developing and less consistent in its attack than at Rockwell (table 2), but undoubtedly hastened the decline of the population. Coccinellids came in slightly later than at the Rockwell field, but at the time of population decline were important in the elimination of the remaining aphids. The schedule of treatment was maintained with more regularity than at Rockwell, and this was evidenced by

Table 2.—Biotic factors influencing populations of *Myzus persicae* developing on sugar beets during the spring of 1958, Kern County, California.

Date	No. Plants Sampled[a]			Per Cent of Plants Sampled[a] with: Live Aphids			Fungus-killed Aphids			Parasitized Aphids			Coccinellids[b]		
	R	C&L	H	R	C&L	H	R	C&L	H	R	C&L	H	R	C&L	H
2/11	400	400	400	<1	0	<1	0	0	0	0	0	0	0	0	0
2/18	400	400	400	2	2	<1	0	0	0	0	0	0	0	0	0
2/25	400	400	160	6	8	4	0	<1	1	0	0	0	0	0	0
3/4	160	400	160	49	9	18	1	1	0	0	0	0	0	0	0
3/11	160	160	160	63	26	16	1	0	0	0	0	0	0	0	0
3/18	160	160	160	90	65	22	2	1	0	1	0	0	0	0	0
3/25	160	160	160	99	90	39	18	4	3	2	0	0	0	0	0
4/2	—	160	—	—	80	—	—	<1	—	—	0	—	—	0	—
4/8	88	160	70	100	98	70	34	53	5	4	0	0	0	0	0
4/15	40	40	40	100	100	100	98	30	0	15	5	5	3	1	3
4/22	40	40	39	100	100	100	95	80	13	30	0	25	0	10	0
4/29	40	40	40	100	100	99	65	40	—	23	8	60	43	5	5
5/6	40	40	40	100	100	38	70	83	0	50	15	13	23	48	10
5/13	40	39	40	56	57	5	15	15	0	18	3	5	28	49	13

[a] R = Rockwell field, North of Argin; C&L = Camp and Lackenmaier field, southeast of Schafter; H = Henson Bros. field, southeast of Bakersfield.
[b] Coccinellid counts include adults, larvae (all instars) and prepupae.

the continual depression of the nymphal aphid population from March 11 until May 6 when treatment was discontinued (fig. 3). Alatoid nymph production reached maximum in the Camp and Lackenmaier field coincident with the peaking of the nymphal population, and this was followed a week later by a noticeable increase in the number of alates per plant found on the untreated plots (fig. 1). Fungus attack apparently was not sufficiently heavy to prevent maturation of the alatoid nymphs and the subsequent within the field alate flight, as was the case in the Rockwell field.

Henson Bros. Field.—In spite of the comparatively early, heavy, and continuous immigration of a late *Myzus persicae* into this field (fig. 1), large colonies of aphids failed to develop on the plants regardless of treatment. The maximum nymphal population attained approximately the level of 20 to 30 aphids per plant. Fungus probably was of little consequence in affecting the population trend in this field, presumably owing to the lack of contiguous suscepts (table 2). Braconid parasites were more noticeable in this field than in either of the others, and because of the relatively few aphids per plant, they affected a greater proportion of the population. Coccinellids were late in developing in the Henson Bros. field, but finally became sufficiently abundant to insure residual population cleanup. Alatoid nymphs never were abundant, presumably because the population density failed to reach the threshold value in the majority of the colonies (table 1). The treatment schedule was comparable to that at the Camp and Lackenmaier field, and it is evident that it suppressed population development quantitatively.

Virus Control.—Initial Sources and Introduction. In spite of the beet-free period and the cultivation of fields which had been previously cropped with beets, the prolonged wet spring favored the rooting of tops and pieces of old beet root, both within fields that were going to be replanted to beets, and fields which were to be fallowed or cropped with something other than beet. Thus there were adequate initial sources of virus, without assuming the existence of weed host virus source plants.

The first evidence of virus introduction into any plot area was on April 2, in the Camp and Lackenmaier field, when one mosaic-diseased plant was found during the aphid sampling period. The following week in the Rockwell field, one mosaic-infected plant and one yellows-infected plant were found. Adjusting for the incubation period of these diseases, it was probably about mid-March when the actual inoculation occurred, which was coincident with the first noticeable rise in alate activity (fig. 1).

Rockwell Field.—Little virus incidence control was obtained in this field. The combination of rain and heavy soils prevented spray application for approximately a month (April), and the nymphal population on both treated and untreated plots increased to an estimated 30 to 40 nymphs per plant. Since yellows had been noted in this field on April 8, failure to control the early April population rise probably was critical as far as virus spread was concerned. The immigrating alatae may have had a greater proportion of inoculative insects and as a result, more foci of infection could have been established during the initial phases of population buildup and dispersal within this field. Evidence for this premise was the delay in the appearance of either yellows or mosaic diseases in the Henson Bros. field, in spite of the fact that initial alate flights occurred approximately at the same time and were at least as heavy as they were in the Rockwell field.

An actual virus disease count was begun on May 28, but was discontinued when it became evident that more than 50% of the plants were diseased either with yellows virus alone or in combination with mosaic, regardless of treatment. The count indicated approximately 68% infection with yellows in the treated plots (566/839) compared with about 80% infection in the untreated plots (776/959). Thus while evidence for less yellows infection in the treated plots could be supported statistically ($\chi^2 = 42.5$, d.f. 1, $p = 0.001$), little in the way of practical control was anticipated.

Camp and Lackenmaier Field.—Yellows was not found in this field until April 29. Incoming alate flights

were lighter and somewhat delayed in comparison with the other two locations, and although the peak aphid populations were far greater in this field than in either of the other two, the regular treatment schedule apparently resulted in a suppression of nymphal populations and prevented the release of a secondary within the field alate flight such as occurred in the check plots (fig. 1). Disease counts were begun on April 30, and continued at approximately weekly intervals until May 20, at which time the data indicated approximately 25% yellows (with or without mosaic) incidence in the treated plots, contrasted with approximately 90% in the untreated areas. This difference in disease incidence was visually evident with the treated strips appearing as green stripes through the field.

A month later, on June 20, a disease count indicated that approximately 100% infection was present in the plot area independent of treatment. Thus treatment delayed but did not prevent virus spread, and it was not effective enough to prevent the eventual swamping of the treated areas with disease presumably because of the close proximity of a large and apparently highly inoculative vector population. An approximation[7] of the difference in the rate of virus spread in the treated and the untreated plots is given in figure 4, assuming a logarithmic rate of increase.

Probably the large alate population which developed on the check plots and which peaked on May 21, was responsible for the disease spreading rapidly over the entire plot area, and accounts for the rather poor fit of the regression line during this period. This conclusion is somewhat speculative, but the gap between the high secondary within field alate population on the check plots and the occurrence of practically 100% infection in the treated plots would be expected, owing to the incubation period of the disease in the inoculated plants.

Henson Bros. Field.—In contrast to the spread of virus yellows in the Wasco and Schafter area, was the situation that obtained in the Maricopa flats area. Here in spite of the relatively early, heavy, and continuous incoming of alate green peach aphids during February, March, and April, populations failed to develop on the beets and virus introduction was minor. The reasons for either of these phenomena are unknown. Virus spread was slow early in the season, for on May 20, disease counts indicated approximately 4% yellows in the treated plots, and about 11% in the untreated ones. A second disease count made on June 16 indicated that the disease in the treated plots had increased to approximately 35%, while in the check plots it was approximately 56%.

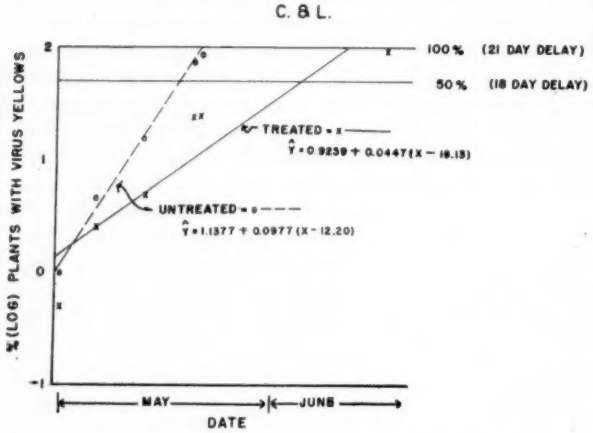

FIG. 4.—Regression lines estimating the rate of virus yellows spread in demeton treated and untreated plots in the Camp and Lackenmaier field of sugar beets during the spring of 1958. The method of estimating the value of the points is given in text.

Even with a low rate of virus introduction by incoming alates, and relatively low aphid populations, evidence for decreasing the incidence of disease (rate of spread) by the use of demeton was demonstrated. But perhaps of more interest was evidence that virus spread continued regardless of the fact that aphid populations on the plants had been reduced to almost undetectable levels by what might be considered feasible sampling methods.

Yields.—The Rockwell plot was harvested August 26, 1958, Henson on September 3 and Camp and Lackenmaier on September 4. The raw data were recorded in pounds (to the nearest 0.5 pound) per 40 feet of 30-inch row. The initial analysis of variance used the figure for pounds of clean beets per 40 feet of 30-inch row (raw weight − tare (bucket + bag) × % clean beets), in order to measure possible effects of treatment and replication on yield. In the analysis the weights were rounded to the nearest pound. The preliminary analysis indicated replication homogeneity in all locations, and therefore the 40 subsample weights from both the treated and untreated plots were used for a t test of significance. This test was also used to judge the evidence for differences in percentage of sugar. The mean yields in tons per acre (mean yield in pounds per 40 feet of row × 0.217) and the per cent sugar for both the treated and untreated plots in each of the three fields are given in table 3.

[7] For purposes of estimation, it was initially assumed that the May 20 count (which included the number of healthy plants as well as a measure of the row distance needed to include 300 plants) could be used as an estimate of the degree to which the more rapid but superficial count of May 19 was reading low. From this percentage, estimates for disease incidence were made for the April 30, May 5, and May 12 counts, and a regression line was calculated, assuming a logarithmic rate of increase. Once the value of b was obtained, the April 30, and May 5 and 19 estimates were recalculated allowing for the 24-hour difference between the May 19 and the May 20 readings, and from these corrected values the final regression line was calculated.

Table 3.—Yields[a] in tons per acre of sugar beets and the per cent sugar obtained in insecticide-treated and untreated plots established in an attempt to control spread of beet yellows virus at three locations in Kern County, California, in 1958.

	Location		
	Rockwell	Camp and Lackenmaier	Henson Bros.
Yield			
Treated	16.8	26.3	37.6
Untreated	16.7	18.5	33.7
L.S.D. at 5% level	2.3	1.9	1.1
Per cent sugar			
Treated	13.3	10.8	12.5
Untreated	12.2	9.5	11.8
L.S.D. at 5% level	0.4	0.7	0.4

[a] Based upon 40 samples. The method of sampling and factors used in the calculations to convert the raw data to tons/acre are given in text.

The evidence for the following conclusions was statistically supportable at a minimum of 5% level of significance. Increased yields in tons per acre of sugar beets were obtained in the treated plots at both the Camp and Lackenmaier and the Henson Bros. fields, but not at the Rockwell field. Increased percentage of sugar was found to be associated with treatment in all locations.

Although it is possible to explain the various results on yield and sugar almost strictly in terms of virus incidence, it would be quite speculative to do so. A number of factors affect the yield and quality of the beet crop, and in these field trials, only a few were subjected to any measurement. In general under conditions of early and relatively heavy infection of beets with mosaic and yellows (Rockwell, 68% versus 80% infection in treated and untreated plots by May 28), lack of treatment was associated with sugar, but not weight loss. It might be assumed under these conditions that failure to attain good control of early virus incidence, plus generally poor cultural conditions, resulted in a loss of experimental sensitivity as far as yield is concerned but still allowed a measurable difference in sugar percentage.

In the situation (Camp and Lackenmaier) where the infection of yellows was delayed by treatment until the beets had reached greater size (25% versus 90% infection in treated and untreated plots by May 28), it was possible to measure a difference both in percentage of yield and sugar. This field suffered from a late attack of leaf spot disease, which caused partial defoliation and stimulated a regrowth. Perhaps this fact is the explanation of the over-all depression of sugar content.

Finally, in the case (Henson Bros.) where the onset of yellows was considerably delayed (4% versus 11% infection in treated and untreated plots by May 28), and the incidence of mosaic was relatively minor, a small difference in yield and sugar occurred. Again these statements are correlative, and the potential cause and effect relationships cannot be deduced from this type of experimentation where many relevant variables have not been assessed.

Hermetia illucens (Linnaeus) as a Factor in the Natural Control of *Musca domestica* Linnaeus[1]

DEANE P. FURMAN,[2] ROBERT D. YOUNG,[3] and E. PAUL CATTS[2]

ABSTRACT

Hermetia illucens (Linn.) was reared in the laboratory from field-collected larvae. Newly hatched larvae required 2 weeks or longer to attain full growth at a temperature of 30° C. in moist standard house fly breeding medium. No evidence of paedogenesis was demonstrated. Larvae displayed little resistance to freezing. The pupal stage lasted from 2 weeks to 5 months at temperatures ranging from 21° to 28° C. Adult flies held at temperatures around 30° C. were active and fed on honey diluted with water or sugar-water solutions. Adults were eurygamous, refusing to mate in cages of 25 cu. ft. volume. Sterile egg masses were deposited.

In combined laboratory cultures of *H. illucens* with *Musca domestica* Linn. the per cent of successful development of house flies decreased as the number of actively feeding larvae of *H. illucens* was increased. This relationship was modified by the total amount of larval medium available. *H. illucens* larvae were not cannibalistic or predacious on house fly larvae, although they ate dead flies.

Field observations over a period from October 1957 through September 1958, in the upper San Joaquin Valley of California, demonstrated adult *H. illucens* activity from April to November, with a peak in late August. Larvae occurred in poultry manure throughout the year, but were relatively inactive during winter months. House flies were very rarely found breeding in manure where *H. illucens* larvae were abundant. Artificial infestation of poultry droppings with *H. illucens* larvae prevented house fly breeding, while adjacent moist control sections became heavily infested with house flies.

On numerous occasions in the past few years the popular press has reported control of the house fly, *Musca domestica* Linn., on poultry farms where manure was naturally infested with larvae of the soldier fly, *Hermetia illucens* (Linn.). Since no experimental data seem to have been published evaluating a possible antagonistic effect between the two species, investigations were initiated in 1957 to clarify the situation.

As pointed out by James (1935), *Hermetia illucens* occurs in the more moist tropical and subtropical regions of the world. It is a relatively common fly in parts of the Southern United States.

In 1926 Copello recorded observations on the biology of *H. illucens* at Buenos Aires, Argentina. He stated that adult flies emerged from spring through the summer and early fall months. Mating commenced during flight. Females characteristically oviposited around the edges of decaying masses of organic debris. Eggs hatched in a few days, but larval development was slow. A single generation was produced each year, with larvae spending the winter in quiescence. Although Copello felt that the flies aided in the sanitation of the environment, he gave particular attention to their habit of ovipositing in cracks of beehives; the larvae subsequently fed on the contents of the hives. He indicated that larvae of *Hermetia* caused perceptible damage only in weak colonies of bees.

LABORATORY TESTS.—*Hermetia illucens* larvae were obtained from poultry manure for laboratory observations

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and culture. In routine culture the larvae were held in glass jars, 10 inches in diameter by 12 inches in height. For each culture 200 to 500 ml. of CSMA (Chemical Specialties Manufacturers' Association) standard fly larval medium (Ralston Purina Company) were used per week, moistening the material to the same degree as in house fly cultures. Additional water was added as necessary so that the medium remained damp but loose. A typical culture medium was demonstrated to contain 52% water by weight. Cultures were held both at room temperature and at a constant temperature of 30° C.

Pupation proceeded satisfactorily in the culture jars. Routinely the pupae were left in the jars and the resulting imagoes removed upon appearance.

Adult stratiomyids were held in two types of indoor cages. The first was an 18-inch screen cubicle held in a 30° C. glass-fronted incubator at a relative humidity of approximately 90%. The second type was a plastic screened cage, 2½ feet square by 4 feet high. Two 100-watt incandescent lamps were placed against the screening at floor level, while a third was placed at the top of the cage. An 18-inch-wide band of cotton gauze, hanging the length of the cage, was maintained as a moist surface, dripping into a screened enamel pan on the floor of the cage. The entire cage was covered with heavy paper to aid in retaining heat. This assembly provided a temperature of approximately 28° to 30° C. at the top of the cage.

Adults in both cages were fed on sugar-water and honey diluted with water; additionally, jars of moist larval culture medium were accessible.

To determine the developmental rate of *H. illucens* larvae, 100 of the smallest field collected specimens, ranging from 1 to 3 mm. long were cultured in an excess of moistened, standard fly-breeding medium held at 30° C. Within 4 days their length ranged from 10 to 15 mm. At the end of 2 weeks they had attained lengths ranging from 25 to 30 mm., and 50% of the remaining 79 viable larvae had pupated. Duration of the larval stage in routine cultures was approximately the same as described above except in those instances in which the food supply was reduced; this reduction in food supply prolonged the period of larval development up to 4 months. Addition of fresh food to a larval culture resulted in a rapid churning of the upper strata of the medium as the larvae worked beneath the surface. Larval activity was also increased by the addition of water to replace that lost by evaporation.

Larvae of *H. illucens* fed voraciously on the bodies of dead larvae or adults but were never observed to attack the living forms. Pupae were not attacked by larvae.

Larvae isolated in glass beakers were destroyed by 30 minutes' exposure to −2° C., but at 2° C., in a thin layer of culture medium, most larvae survived 24 hours' exposure; 60 hours' exposure under the latter condition was uniformly lethal.

Repeated rearing of known numbers of larvae produced no evidence that multiplication by paedogenesis occurred.

The pupal stage lasted for approximately 2 weeks under routine culture methods as well as in a detailed study carried out at room temperatures ranging from 21° to 28° C. However, occasional specimens exhibited a prolonged pupation period; *i.e.* 5 of 79 pupae produced adults after 2 to 5 months. No sex-associated difference in the length of the developmental period was noted.

Adult *Hermetia illucens* were demonstrated to be positively phototropic. At 30° C. the flies were quite active in the culture cages. When removed to room temperatures appreciably below 30° C. they became sluggish and were easily captured by hand. No mating was noted in either small or large cages. However, females deposited sterile egg masses. Typically, oviposition took place through the screen mesh of the cages. The cigar-shaped, yellowish ova were deposited singly in rapid succession to form masses of 500 to 1,000 eggs. Adult flies lived for 2 weeks or more under routine culture conditions.

Several laboratory experiments were conducted with simultaneous cultures of house flies and stratiomyids in an effort to determine the effect of the associations on house fly breeding. The first of these was designed to test the hypothesis that cultures supporting heavy populations of growing *Hermetia illucens* larvae will fail to produce house flies following exposure to gravid, adult, female house flies.

Two 600-ml. beakers, each containing 300 ml. of dampened standard fly medium were exposed side by side for 3 days to the same population of adult house flies. One beaker contained 500 *H. illucens* larvae of mixed sizes. The beakers were removed from the house fly cage and held for 3 weeks at room temperature. No food was added subsequently to these cultures but it was necessary to moisten the medium occasionally with distilled water. Approximately 1,000 robust house fly adults emerged from the beaker containing no stratiomyid larvae. The other, which contained *H. illucens* larvae, produced no house flies.

A variation of the above experiment was conducted in which a tablespoonful of fresh, standard larval medium was added to each culture every third day. During the 3-week observation period more than 500 house flies were produced in the control beaker, while only 9 were produced from the beaker containing *H. illucens* larvae.

In order to determine more precisely the quantitative effect of increasing numbers of active *H. illucens* larvae of all ages upon *M. domestica* in simultaneous culture, the following experiment was conducted. Four finger bowls, each containing 100 ml. of moist, standard fly-breeding medium, were seeded with viable house fly eggs. Known numbers of *H. illucens* larvae were added to three of the bowls. The cultures were held at 27° C. The results of the test are given in table 1. The data demonstrate a very high degree of control obtained in each of the test cultures; in the culture containing only 200 stratiomyid larvae, however, a few house fly adults emerged, while no house fly larvae pupated in either of the cultures containing greater numbers of stratiomyids.

The objective of the following experiment was to test the possibility that old culture medium previously used by *H. illucens* larvae contains a substance which may materially decrease house fly breeding. Approximately 2,000 house fly eggs were placed in each of three jars containing media as follows: (1) 2,000 ml. of moist standard fly medium; (2) 1,000 ml. of moist standard fly medium and 1,000 ml. of old stratiomyid culture medium; (3) as in "2" but with the addition of 500 full grown larvae

Table 1.—Emergence and survival of *Musca domestica* in simultaneous cultures with *Hermetia illucens* larvae.[a]

	Hermetia illucens				*Musca domestica*					
	Initial Number	Final Number			Initial Number	Final Number			Emergence (%)	Survival all Stages (%)
CULTURE	Larvae	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults		
Control	0	0	0	0	100	0	2	70	70	72.0
A	200	60	138	1	200	0	0	14	7	7.0
B	400	241	157	0	228	13	0	0	0	5.7
C	600	350	245	5	223	11	0	0	0	4.9

[a] Each culture contained 100 ml. standard fly medium; duration of observations, 19 days.

of *H. illucens*. The adult house fly emergence from the three jars was quantitatively similar, although emergence from the jar containing stratiomyids required 4 days longer than from the other cultures. These data indicate that house flies were not appreciably harmed by substances produced in the breeding substratum by *H. illucens*. Furthermore, larval *M. domestica* developed normally, although slowly, in the presence of relatively inactive, mature or nearly mature *H. illucens* larvae.

The possibility that *H. illucens* larvae might be predacious upon *M. domestica* was investigated briefly. Larvae of both species held together in glass beakers for several hours exhibited no predacious activity. Similarly, known numbers of house fly larvae survived several days' exposure with starved *H. illucens* larvae when held in test tubes containing only a small amount of standard fly-breeding medium.

Stratiomyid larvae were observed feeding on dead house fly adults, as well as on presumably dead pupae. Normally house fly larvae migrate out of moist culture medium to pupate. In combined laboratory cultures of both species, house fly pupae were frequently buried through action of the stratiomyid larvae. From 46 to 54% mortality of house fly pupae was recorded in a series of cultures in which known numbers of pupae were added to vigorous cultures of *H. illucens* larvae.

Mortality of house fly pupae in combined laboratory cultures should not be interpreted as typical of that occurring under field conditions; on the other hand it is not of such magnitude as to account for the very high degree of control of house fly breeding reported in the foregoing joint culture experiments.

FIELD OBSERVATIONS.—Natural infestations of poultry manure with *H. illucens* were observed from October 1957 through November 1958. The area selected for study was the upper San Joaquin Valley of California, in the Modesto-Turlock vicinity. This is in a region of winter rains, and hot, dry summers. In the Modesto area where normal annual precipitation averages about 11 inches, the 1957–58 season was marked by unusually heavy rains, totaling almost 27 inches in the period from October 1957, through September 1958. Most of the rain occurred from November through April, with none from June through August. The monthly mean of the daily maximum temperatures dropped rapidly from 23° C. in October to 11.7° C. in December. By April it had again risen to 23° C. In July through September 1958 the figure was continuously over 32° C.

Adult *Hermetia illucens* gradually disappeared during October, but occasional adults were observed well into November 1957. They were absent from December through March, appearing first in small numbers at the end of April. Populations of adult flies increased gradually during the following months, reaching a peak during August. In late August and early September they became so numerous on some farms as to constitute a nuisance, but since they rarely entered homes the annoyance was minimal. In mid-September the adult population of soldier flies was somewhat reduced, with activity most pronounced on hot days.

Larvae of *H. illucens* were observed in poultry manure throughout the year, but during the winter months growth and activity were greatly reduced. Although living pupae were present during winter months, their emergence was apparently delayed until the advent of warm, spring weather. Early instar larvae were very numerous during June, indicating earlier oviposition activity, but egg masses also were laid in nature during subsequent warm weather.

Heavy infestations of *H. illucens* larvae were most frequently observed in relatively moist poultry droppings. In such situations fresh droppings were worked into the mass of manure by the soldier fly larvae as fast as they were deposited during the warm season. The larvae usually occurred in an almost solid layer in the top 2 or 3 inches of manure, continually turning over the surface layer of droppings. In cold weather the droppings accumulated, relatively undisturbed by the inactive stratiomyids.

During the warm season *Musca domestica* very rarely was found breeding in areas of poultry droppings where *H. illucens* larvae were abundant. However, house fly breeding was common on poultry farms where the distribution of soldier fly larvae was uneven. The latter migrated only slowly from foci of heavy infestation to adjacent areas, and it was in such lightly infested sites that house flies continued breeding.

Although *H. illucens* larvae frequently were found breeding in semi-liquid manure, tremendous populations also were found in merely moist droppings. The effect of *H. illucens* larvae upon the drying rate of poultry manure has not been determined. On the one hand they prevent the buildup of manure cones under single or double bird cages, thus perhaps decreasing the evaporation rate; on the other hand they continually agitate and perforate poultry droppings, probably resulting in a drying effect. Observations on laboratory cultures of soldier flies in artificial medium containing approximately 50% water by weight, as described above, indicate that a loose,

friable surface layer develops, with the old medium forming a moist, semisolid substrate.

FIELD TESTS.—Experimental plots were established in early and late July 1958, to determine the effect of artificial introduction of *H. illucens* larvae on subsequent house fly breeding in poultry droppings. Additionally, information on the effect of moisture on soldier fly breeding was sought.

Plots for experimental management studies consisted of alternate, 5- by 8-foot sections of manure under a double row of two-bird cages. Cages were protected from sun and rain by roofing, but walls were of the open type. House fly breeding occurred on the moist tips of manure cones in two adjacent poultry houses, and an almost pure culture of *H. illucens* flourished under a third house. The manure in the test plots was quite dry, since the housing was newly occupied and the warm, dry climatic conditions were adequate to dehydrate the fresh droppings as they accumulated. Consequently there was very little fly breeding in the test plots at the start of management procedures.

Three plots were merely moistened to a mushy consistency by adding water. Three plots were treated similarly but with the addition to each of half a wheelbarrow-load of moist manure heavily infested with *H. illucens* larvae. Two additional plots were left dry, but to each was added a similar load of *H. illucens* infested manure; the infested manure was covered with dry droppings on one plot but placed over the dry material on the other.

The results are given in table 2. House fly breeding was immediate in plots treated only by moistening. Plots moistened and infested with *H. illucens* larvae exhibited no house fly breeding. *H. illucens* larvae became established in the relatively dry manure plots when artificially introduced, but no house flies established breeding. Adult soldier flies were attracted to plots where freshly turned manure infested with their larvae was exposed. Oviposition by the soldier flies apparently occurred, since large numbers of newly emerged larvae were observed 3 weeks later in such manure while nearby droppings remained negative for the larvae.

Since observations indicated that larval activity and population increase of *H. illucens* were somewhat inhibited by increasingly dry conditions, an experiment was designed to determine the effect of adding moisture to relatively dry manure, moderately, but uniformly, infested with soldier fly larvae. A 30-square-foot plot of manure selected for the experiment was shaded from all but the early morning sunlight. Except for a dry, hard, surface layer, the manure had been reduced to a rather loose, friable consistency by soldier fly larvae. At 1- and 3-week observation periods following addition of water, the *H. illucens* larval activity was greatly increased, with thorough turnover of new droppings. Soldier fly larvae in adjacent areas of dry manure remained relatively quiescent, although at moderately dense population levels. No house flies became established in either situation, although their larvae were common in moist droppings under an adjacent house.

DISCUSSION.—The evidence reported here is considered as strong support for the hypothesis that *Hermetia illucens* may serve as a significant factor in the natural control of house fly breeding. Field observations on the beneficial effects produced by *H. illucens* were restricted to an area of high summer temperatures and low relative humidity, coupled with relatively mild winters. Scarcity of the stratiomyid in the more temperate coastal region of central California seems indicative of an environment unsuited for development of the dense larval populations required for effective control of house fly breeding.

In the San Joaquin Valley of California, *H. illucens* larvae thrive in moist poultry droppings during the warm season under the conditions most favorable for an explosive breeding rate of house flies. In the presence of a dense population of soldier fly larvae however, the more rapidly developing house fly larvae are unable to compete successfully, appearing to be starved out by the stratiomyids. There is no evidence that larvae of the latter are anything but scavengers.

Where *H. illucens* larvae are present in large numbers in poultry droppings their effectiveness for control of house fly breeding may be enhanced by spreading manure

Table 2.—Effects of management procedures on house fly breeding in poultry droppings.[a]

MANAGEMENT PROCEDURE	PLOT NUMBER	POST-TREATMENT (WEEKS)					
		One		Two		Three	
		M. domestica	*H. illucens*	*M. domestica*	*H. illucens*	*M. domestica*	*H. illucens*
Manure moistened	1	Abundant	None	—	—	Few[b]	None
	2	Abundant	None[c]	None	Abundant	—	—
	3	Abundant	None[c]	None	Abundant	—	—
Manure moistened; *H. illucens* larvae introduced	4	None	Abundant	—	—	None	Abundant
	5	None	Abundant	None	Abundant	—	—
	6	None	Abundant	None	Abundant	—	—
H. illucens larvae spread over dry manure	7	None	Moderate	None	Abundant	—	—
H. illucens larvae introduced under dry manure	8	None	Moderate	None	Abundant	—	—

[a] All poultry droppings dry, with little fly breeding before management started.
[b] Manure quite dry.
[c] *H. illucens* artificially introduced.

containing the stratiomyids over droppings infested with house fly larvae. Where dry cones of droppings have developed, *H. illucens* is relatively ineffective in controlling house fly breeding on the moist tips of the cones, hence manure cones should be removed or leveled before adding soldier fly larvae. An active, dense population of soldier flies will prevent further cone formation under wire-floored cages during the warm season.

In a sense, the use of soldier flies in the natural control of house flies and related species may be considered as trading one pest for another. The nuisance value of *H. illucens* is minimized, however, through the fact that it is a slowly maturing fly in all except very warm weather, hence the flies are present primarily as larvae for much of the year. Furthermore, the adult flies, which may become abundant for a short time in the summer, rarely enter houses.

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The Use of the Plum Curculio in Toxicological Studies[1]

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ABSTRACT

A method of insecticide evaluation using laboratory-reared plum curculio, *Conotrachelus nenuphar* (Hbst.), is reported. Test insects from the laboratory culture responded to the various treatments similarly to field-collected ovipositing beetles but dissimilar to nonovipositing beetles.

The evaluation procedure was designed to disclose inherent toxicity and residual effectiveness under natural weathering conditions. Inherent toxicity was determined by topical application and by feeding. In the former, one microliter droplet of insecticide in acetone was applied to the dorsal thoracic area of test insects. Feeding tests were conducted by confining individual ovipositing beetles on treated immature apples. Results of feeding tests were based on reduction in feeding and oviposition as well as mortality. Residue tests were based on mortality of beetles caged on prune foliage collected from the field at intervals following spraying.

Measurements of inherent toxicity can be conducted in the laboratory without regard to season. The evaluation procedure reported is useful in preliminary screening and in accounting for results under practical conditions.

A method for continuous rearing of the plum curculio, *Conotrachelus nenuphar* (Hbst.), has previously been reported (Smith 1957b). The present paper describes a method for employing the laboratory-reared insects in toxicological studies and reports results with numerous insecticides.

The procedure used to measure toxic action involved two phases. The first was a measure of inherent toxicity by both topical application and feeding. The second phase measured the toxic life of spray deposit under natural weathering conditions. The factors accounting for control under practical conditions, namely, inherent toxicity and residual persistence, are thus incorporated in the evaluation procedure.

In order to relate experimental results using laboratory-reared insects to practical control it was necessary to compare the response of the laboratory strain with naturally occurring beetles. Parathion was selected as the standard insecticide for this comparison. Two classes of naturally occurring beetles in addition to the laboratory strain were employed. The sources and characteristics of the three classes of insects are as follows:

Laboratory Strain.—These are the progeny of the southern strain obtained in 1949 and reared continuously in the laboratory. This class is characterized by the absence of reproductive diapause and higher rates of feeding and oviposition than naturally occurring beetles.

Northern Hibernated Beetles.—These are collected locally from unsprayed host trees in June. They are single brooded but oviposit following the termination of reproductive diapause by hibernation.

Northern Unhibernated Beetles.—These were reared in the laboratory from northern hibernated beetles and are comparable to the local "summer brood." They are characterized by reproductive diapause and low rates of feeding.

Further discussion of the characteristics of these classes is contained in the references cited.

TOPICAL TREATMENT.—Technical insecticide dissolved in acetone was prepared in concentration series and a dosage of 1 microliter applied to the dorsal thoracic area of test insects. The micrometer syringe employed to deliver measured droplets was adapted from the model designed by Dutky (1942) (fig. 1). Fifty insects were generally treated at each concentration. It was not necessary to anaesthetize the insects before treatment although anaesthesia with carbon dioxide had no adverse effect. Following treatment, insects were caged on untreated green apples and mortality counts made daily. Dosage-mortality curves were plotted using the method of Bliss (1935). Values were expressed as milligrams per gram of body weight.

Before standardizing experimental procedure, tests were conducted to determine the influence of age, sex, strain, and site of treatment on susceptibility, using parathion as a standard. Droplets were applied to the head, thorax, and abdomen. The LD_{50} value for treatments to the abdomen was about half the concentration required for treatment to the head and thorax. This is possibly due to greater penetration through the flooding

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FIG. 1.—Dutky-Fest microsyringe used in topical treatments of the plum curculio with one microliter dosages of insecticide in acetone.

of spiracles and coverage of the thin body wall beneath the elytra. The dorsal area of the thorax was selected as the site for routine treatment because of the ease in manipulating the insects and greater consistency in results.

LD_{50} values for males and females of the laboratory and northern strains treated with parathion were as follows:

Class	*Sex*	*LD50 Mg./GM.*
Northern hibernated	Female	0.0071
Northern unhibernated	Female	.0074
Northern hibernated	Male	.0074
Laboratory	Female	.0066
Laboratory	Male	.0067

The variations shown are not significant. The values for different sexes are based on actual average weights rather than on assumed weight of 15 milligrams per insect as is routinely used. The average weights previously reported for laboratory males and females are 14.7 and 15.4 mg. respectively (Smith 1957b). Differences in susceptibility between the various classes of females were not disclosed despite striking differences in behavior. This fact suggests that no physiological differences in susceptibility occur between the classes although behavioristic differences, particularly feeding rate, might account for differences by other methods of testing. The LD_{50} value for beetles 2, 26, and 85 days of age were not significantly different.

Based on these findings differences in age, sex, and strain are not critical in topical application tests. However, as an abundant supply of laboratory-reared insects has been available, mixed sexes between 3 and 6 weeks of age have been used. The dosage-mortality curves for several insecticides by topical application are shown in figure 2. The most toxic of these was Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) followed by Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical). DDT is not shown in figure 2 but was found to have an LD_{50} of approximately 4.0 mg. Most of the materials fall within the 10-fold dosage range of 0.001 to 0.01 mg. This range is less than might be expected based on differences in control under practical conditions.

FEEDING TESTS.—Females were confined individually under inverted jelly glasses supported on screen racks. A treated apple approximately 1 inch in diameter was exposed to each female for 1 day, after which, with the aid of a binocular microscope, it was removed and examined for feeding punctures and eggs. In addition, daily counts also were made on mortality and tests terminated after 10 days. Twenty females were generally employed at each concentration. Counts were made for several days prior to treatment to establish the rate of feeding and oviposition, and in addition, counts on untreated insects were continued for the duration of the test. Apples were treated by spraying on a turntable to the point of run-off, using a DeVilbiss paint sprayer operated at 20 lbs. pressure. The toxicant was employed as formulated for commercial use, usually wettable powder, and concentration expressed as parts per million of toxicant in the spray mixture.

The northern hibernated insects used in the compara-

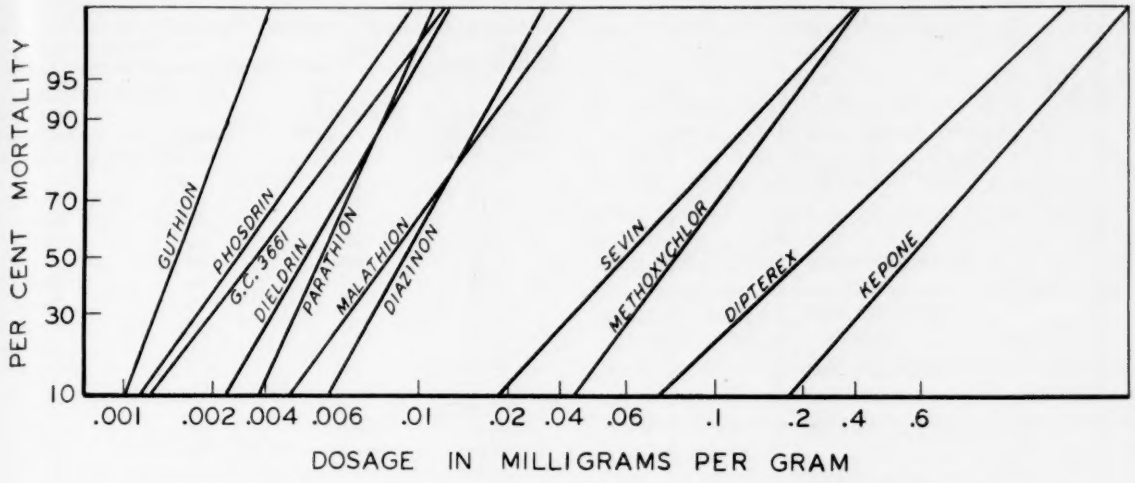

FIG. 2.—Dosage-mortality curves for the toxicity of insecticides to the plum curculio by topical application.

tive study were collected from host trees on June 4, 1957, when oviposition was near the peak. Sixty ovipositing females of each class were observed for 2 days prior to testing to establish rates of feeding and oviposition. Average oviposition rates (80° F.) were 10.4 and 9.8 eggs per female per day for the laboratory and northern females, respectively. The record of mortality, feeding, and oviposition, and reduction over untreated insects is shown in table 1. The ratio of eggs to feeding punctures is also shown.

The response of both classes of ovipositing females is essentially the same. At the lower dosages differences in mortality suggest that the field-collected females are slightly more susceptible, but precise LD_{50} values cannot be deduced from these limited data. From a practical point of view the response of these two classes is sufficiently similar to permit valid comparisons. The results using nonovipositing females are strikingly different. The erratic results obtained with this group indicate that they were ill-suited to tests of this type.

The data shown in table 1 might also be cited to illustrate several generalities with respect to the toxicology of insecticides to the plum curculio. At concentrations well above the lethal threshold, mortality does not occur before some injury by feeding and oviposition takes place. Thus, control expressed as per cent mortality is slightly higher than control expressed as reduction in feeding and oviposition. Among lethal dosages a quantitative relationship exists between concentration and injury although the range is not great. Such injury occurs chiefly on the first day of exposure when feeding and oviposition are 50% or more of normal. The ratio of eggs to feeding punctures is not greatly altered during the course of such tests.

To further explore the relationship between concentration of toxicant and injury before death, several insecticides were tested in concentration series ranging from that employed in practice to sublethal levels. These results are presented in condensed form in table 2. The relationship cited earlier in the case of parathion is seen to apply to the other materials as well. In no instance did the concentration employed in practice entirely eliminate feeding and oviposition but the extent of injury at lethal levels was about equal for all materials tested.

The reduction in injury reported in table 2 is accounted for by both death and subnormal activity of survivors. The rates of feeding and oviposition among survivors of several selected tests are shown in table 3. With all materials, injury by survivors is less than normal but the reduction is not so great as with lead arsenate (Smith 1957a). The feeding and oviposition response is essentially the same for survivors of both chlorinated hydrocarbons and organophosphates. However, precise comparisons between materials in sublethal ranges are not readily made because of experimental difficulties. In any event, it seems likely that with synthetic organic insecticides sublethal effects do not become a major factor in control under practical conditions. Thus, having once established the general relationship between lethal dosages and injury, mortality counts alone might be employed as the criteria of effectiveness, thereby reducing the time required for routine feeding tests.

While such data as presented in table 2 are insufficient for the calculation of precise dosage-mortality curves, relative values may be interpolated by inspection. A summary of LD_{50} feeding values deduced in this manner, together with the ratio of this value to concentration recommended in practice, is given in table 4. This ratio is useful in comparing materials employed at different concentrations and it is also broadly indicative of the margin of loss permissible by weathering. This relationship is well illustrated in the case of Guthion and methoxychlor. The LD_{50} values of these materials differ by 50-fold but the less toxic material, methoxychlor, is employed at 5 times the concentration of Guthion, thus reducing the difference in toxicity rating to 10-fold.

For convenience, the concentration of toxicant employed in feeding tests has been expressed as parts per million in the spray mixture. The use of this criterion in quantitative comparisons presupposes equal residual deposit by various materials at a given concentration.

Table 1.—Total oviposition, feeding and mortality of laboratory and field plum curculio females exposed for 10 days to apples sprayed with various concentrations of 15% parathion wettable powder.[a]

Day	Laboratory												Northern Hibernated										Northern Unhibernated									
	Concentration (p.p.m.)												Concentration (p.p.m.)										Concentration (p.p.m.)									
	100		80		60		40		20		Check		100		80		60		40		20		Check		100		80		60		40		20		Check	
	FP[b]	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E	FP	E
1	73	43	85	48	111	71	95	56	162	93	188	98	82	47	112	65	116	50	124	57	155	79	160	85	2	6	15	1	1	11				
2			3	1	12	2	49	20	204	112	214	118					19	5	20	7	177	74	235	114	11	10	10	9	15	13				
3					2	0	84	28	124	46	182	85					2	0	22	4	87	34	136	71	0	8	3	13	2	7				
4					1	0	121	27	117	52	235	92					10	10	16	1	111	47	141	80	31	0	8	16	2	1				
5							111	36	115	23	261	108					27	5	15	1	105	17	172	81	22	15	10	9	3	12				
6							107	35	130	37	259	108					18	1			138	34	164	79	4	13	16	15	3	8				
7							75	20	164	88	283	119									81	18	172	79	8	26	35	22	1	14				
8							88	4	140	33	229	105									89	14	178	91	21	52	44	27	2	16				
9							64	18	138	39	256	110									85	16	152	74	17	28	40	24	0	4				
10							41	14	171	35	210	97									78	12	175	87	27	27	38	39	3	29				
Total	73	43	88	49	126	73	835	258	1,465	558	2,317	1,040	82	47	112	65	192	71	197	70	1,106	345	1,685	841	143	185	219	175	32	115				
Ratio[c]	1:1.7		1:1.7		1:1.7		1:3.2		1:2.6		1:2.2		1:1.7		1:1.7		1:2.7		1:2.8		1:3.2		1:2.0									
Reduction (%)	97	96	96	95	94	93	64	71	37	46			95	94	93	92	89	91	88	91	34	59										
Mortality (%)	100		100		100		70		40		0		100		100		100	100	60		0				10	20	0	0	10	0				

[a] Ten females employed at each concentration.
[b] FP—Feeding punctures; E—Eggs.
[c] Ratio of eggs to feeding punctures.

Table 2.—The mortality and reduction in feeding and oviposition of female plum curculio feeding on various concentrations of insecticide.[a]

Concentration (P.P.M.)	Per Cent Mortality	Per Cent Reduction	
		Feeding	Oviposition
	Guthion		
360	100	99	99
180	100	99	99
90	100	98	98
45	100	94	95
25	100	80	83
20	100	66	75
15	100	65	72
10	70	39	52
5	10	9	9
	Parathion		
360	100	98	98
180	100	97	98
90	100	95	96
80	100	95	93
60	100	96	95
40	70	64	71
20	40	37	46
	Methoxychlor		
1,800	100	98	98
1,200	100	98	98
1,000	100	98	98
800	100	95	97
600	95	92	95
400	60	82	92
	Dieldrin		
300	100	98	97
200	100	98	97
100	100	96	95
75	100	95	95
50	100	93	93
25	85	85	89
20	70	70	79
15	40	56	79
	DDT		
1,200	65	57	80
600	45	39	63
300	20	33	51
150	10	13	18

[a] Highest concentration is that employed in practice.

Table 3.—The influence of sub-lethal dosages of insecticide on rate of feeding and oviposition by plum curculio surviving 10-day feeding test.

Toxicant	Concentration (P.P.M.)	Per Cent Survival	No. of Survivors	Per Cent of Normal	
				Feeding	Oviposition
Methoxychlor	400	40	8	33	10
	200	75	15	45	27
DDT	600	25	5	76	46
	300	80	16	78	54
	150	90	18	100	85
Dieldrin	25	15	3	51	28
	12	50	10	91	66
Parathion	40	30	6	69	49
	20	60	12	85	49
Malathion	100	30	6	36	17
	50	90	18	71	61

Table 4.—Estimated LD_{50} values of insecticides to the plum curculio by feeding and ratio of LD_{50} value to concentration employed in practice.

Material	Estimated LD_{50} (P.P.M.)	Concentration Employed in Practice (P.P.M.)	Ratio LD_{50} to Practical Concentration
Guthion	7	360	1:51
G. C. 3661	10	600	1:60
Dieldrin	18	300	1:17
Parathion	30	360	1:12
Diazinon®[a]	30	600	1:20
Kepone	50	1,200	1:24
Malathion	75	600	1:80
Dipterex	150	3,600	1:24
Heptachlor	200	600	1:30
Methoxychlor	350	1,800	1:50
DDT	750	1,200	1:20
Sevin®[b]	90	1,200	1:13

[a] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[b] 1-naphthyl-*N*-methyl carbamate.

Only in the case of parathion was actual deposit determined. These results are as follows:

Concentration[a] *in spray (p.p.m.)*	*Deposit*[b] */μg./100 cm.²*
100.0	75
50.0	37
25.0	17
12.5	9

[a] Toxicant formulated as 15% w.p.
[b] Residue determinations made by A. W. Avens, New York State Agric. Expt. Sta., Geneva.

The expected relationship between concentration and deposit is seen to apply. It is likely that a similar relationship applies for other materials as well but that various formulations deposit at different rates. In such an instance some error is likely involved in the use of concentration as an indirect measure of deposit. Limited evidence based on residue studies under field conditions (Smith *et al.* 1956) suggests that the margin of error is not great. The LD_{50} value for parathion by feeding is 27 mg. per 100 square centimeters based on the residue determination presented above. This value is in essential agreement with the data just cited for parathion residues on prune foliage.

RESIDUAL EFFECTIVENESS.—A single spray application was made to prune trees in the conventional manner, and at intervals thereafter branches bearing fruits were brought to an insectary and placed under cylindrical screen cages (fig. 3.). The stems of branches were kept in water to prevent wilting. Branches collected early in the season before growth was completed remained in satisfactory condition for a week or more. Two or more replicates of 20 insects each were used for each test and mortality counts were made daily. The northern hibernated beetles used for comparison were collected from host trees during the last 10 days of June 1958 and were somewhat beyond the peak of oviposition. The time-mortality curves for the three classes of insects exposed to parathion residues are shown in figure 4. The 1-day-old residue resulted in complete kill with similar time-mortality curves. Thereafter, differences in the three classes were apparent. The response of the northern

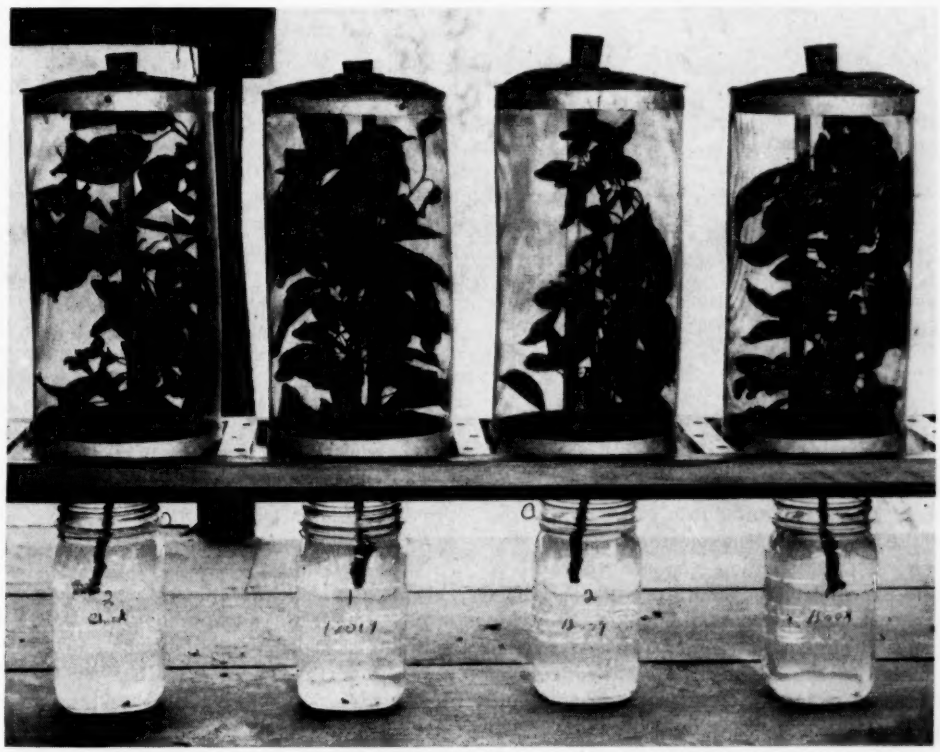

FIG. 3.—Method employed in evaluating the effectiveness of insecticide residues on prune foliage against the plum curculio.

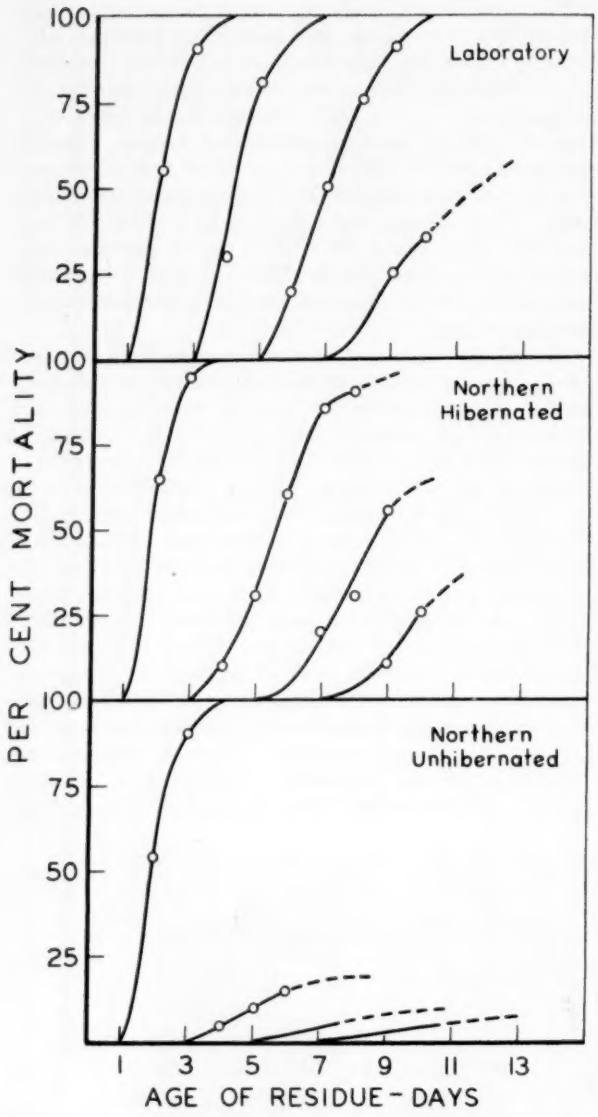

FIG. 4.—Time-mortality curves for laboratory and naturally occurring plum curculio exposed to parathion residue on prune foliage.

unhibernated beetles was strikingly different, showing very low mortality from 3-, 5-, and 7-day residues. The northern hibernated beetles responded similarly to the laboratory beetles although mortality was lower. These differences likely reflect the lower rate of feeding of the unhibernated beetles. Had the northern hibernated beetles been collected earlier their response would likely have been more like that of the laboratory beetles. Even so, it is apparent that the response of the two egg-laying classes is sufficiently similar to permit valid comparisons while unhibernated beetles are unsatisfactory for tests of this type. The results of tests with laboratory beetles and other insecticides are shown in figure 5. Striking differences in various materials are observed. Some, such as Phosdrin, are characterized by high initial kill with little residual effectiveness. Others, such as DDT, are relatively ineffective from the outset, suggesting low inherent toxicity. Materials such as Guthion and General Chemical 3661 (diethyl-1,3-di (carbomethoxy)-1-propen-2-yl-phosphate) possess both rapid speed of action and residual effectiveness. Characteristic differences between chemical groups are not disclosed although in general the thiophosphates show greater speed of action and sharper break in effectiveness than chlorinated hydrocarbons. The effectiveness of some materials as established in practice is not readily apparent from these results. This is best illustrated by methoxychlor, which appears to be slow in its toxic action. Few materials provide complete effectiveness for a period of 10 days, the normal interval between field applications in practice.

By contrast with feeding tests some materials provide complete mortality before feeding or oviposition occurs, as disclosed by inspection of fruits on caged branches. Apparently lethal dosages are acquired by continuous contact with sprayed surfaces such as bark, leaves, and fruits. This difference could possibly be explained by the higher residue deposits on bark as compared with leaves and fruit, at least in the case of parathion (Smith & Avens (1954). In some cases the slope of time-mortality curves reveals the period in which lethal dosages are acquired without feeding. This is illustrated by Guthion residue at 1 and 3 days (fig. 5), which results in a very steep curve and no feeding injury. Thereafter mortality is associated with some feeding and the slope diminishes.

Precise values for residual effectiveness cannot be established as can be done in feeding and topical application tests. In addition, the residue test is subject to numerous variables such as temperature, rain, wind, and sunlight, which are not common to the two tests conducted in the laboratory.

Desirable features of the feeding and residual tests may be combined in still another test in which naturally weathered fruits are collected from the field at intervals and exposed to individual females in the laboratory. As these tests required the holding of fruits in the laboratory, apple was employed as the host fruit rather than prune because of its superior keeping qualities. Tests of this type were conducted with Wealthy apples, sprayed June 27, 1957. Residual effectiveness was likely influenced by rainfall on June 29 and thereafter, but the data are useful for comparative purposes. The standard residual test (caged branches) was conducted concurrently with laboratory feeding tests and results are reported in table 5.

In general, good agreement exists in time-mortality relationships for the two methods. The expected parallel between mortality and reduction in feeding also followed. It is likely that this modification of the feeding test would prove useful in assaying residual effectiveness under practical conditions.

None of the tests conducted takes into consideration the possible influence of surface moisture in the form of dew and rain. In related studies with lead arsenate, imbibing of contaminated surface moisture was found to be an important factor (Smith 1954).

A summary of results by the three types of tests is given in table 6. There is reasonable agreement between contact and feeding toxicity based on comparison with Guthion. This fact is not surprising, as both tests are measures of inherent toxicity aside from the matter of

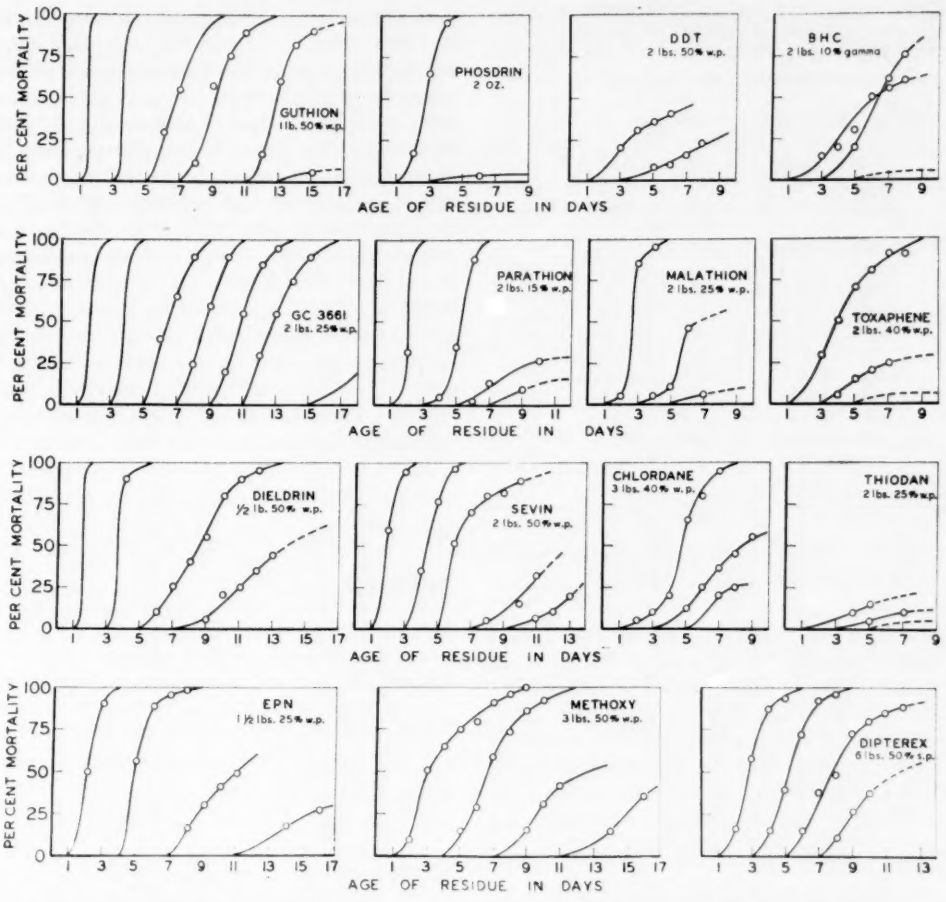
PER CENT MORTALITY
AGE OF RESIDUE IN DAYS
GUTHION 1 lb. 50% w.p.
PHOSDRIN 2 oz.
DDT 2 lbs. 50% w.p.
BHC 2 lbs. 10% gamma
GC 3661 2 lbs. 25% w.p.
PARATHION 2 lbs. 15% w.p.
MALATHION 2 lbs. 25% w.p.
TOXAPHENE 2 lbs. 40% w.p.
DIELDRIN ½ lb. 50% w.p.
SEVIN 2 lbs. 50% w.p.
CHLORDANE 3 lbs. 40% w.p.
THIODAN 2 lbs. 25% w.p.
EPN 1½ lbs. 25% w.p.
METHOXY 3 lbs. 50% w.p.
DIPTEREX 6 lbs. 50% s.p.

FIG. 5.—Time-mortality curves showing residual effectiveness of insecticides to the plum curculio.

penetration. Exceptions occurred in Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) and Kepone® (1,2,3,5,6,7,8,9,10,10,decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one), which are strikingly more toxic by feeding than topically. Presumably these materials do not readily penetrate the body wall but exert toxicity after gaining entry by way of the digestive tract. This feature of Dipterex has been previously cited by Fukuto (Metcalf 1957). Several toxic materials lacked residual effectiveness.

DISCUSSION.—The toxicological features which account for effectiveness of insecticides in control of the plum curculio have been considered. These are inherent toxicity and residual effectiveness. The latter is essential

Table 5.—Comparative effectiveness of insecticide residues to plum curculio caged on branches in the insectary (residue test) and fed on naturally weathered fruits in the laboratory.[a]

MATERIAL (2 LB. W.P.)	AGE OF RESIDUE (DAYS)	INSECTARY CAGE TESTS — Per Cent Mortality After (Days)							LABORATORY FEEDING TESTS — Per Cent Mortality After (Days)							REDUCTION IN FEEDING[b] (%)
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	
Parathion (15%)	1	100							100							98
	3	5	10	35	40	45	55	—	30	50	60	70	80	—	90	61
	6	0	0	5	15	—	—	—	0	0	30	—	—	—	—	25
Sevin (25%)	1	50	65	85	95	—	—	—	50	70	80	—	—	—	—	86
	3	10	20	45	60	80	—	—	0	20	30	40	50	60	70	34
	6	0	0	0	0	15	—	—	0	0	0	10	—	—	—	11
Kepone (50%)	1	50	75	95	—	100			80	90	—	100				96
	3	0	25	45	65	85	90	100	10	—	60	90	—	—	100	84
	6	0	0	5	40	50			0	0	30	70	90	—	—	64

[a] Wealthy apples treated June 27, 1957.
[b] Based on reduction over untreated females. Thirty females averaged 10.5 feeding punctures per day.

Table 6.—The toxicity and residual effectiveness of insecticides to the plum curculio in relation to Guthion.

Material	Toxicity by Topical Application: LD 50 (mg./gm.)	Topical Application: Ratio to Guthion	Feeding: LD 50 (p.p.m.)	Feeding: Ratio to Guthion	Estimated Residual Effectiveness (Days)
Guthion	0.002	1:00	7	1:	10
Phosdrin	.003	1:1.5	—	—	1
GC-3661	.004	1:2.0	10	1:1.4	10
Dieldrin	.005	1:2.5	18	1:2.5	6
Parathion	.006	1:3	30	1:4.3	3
Malathion	.01	1:5	75	1:10	2
Diazinon	.01	1:5	30	1:4.3	3
Sevin	.06	1:30	90	1:13	5
Methoxychlor	.09	1:45	350	1:50	5
Dipterex	.26	1:130	150	1:21	5
Kepone	.6	1:300	50	1:7	7
DDT	4.0	1:2,000	750	1:107	0

because, in practice, control is obtained by maintaining effective residues during the several weeks of adult migration to host trees.

Inherent toxicity is revealed by feeding and topical application tests. The latter is not an infallible guide to toxicity as it likely reflects properties of penetration of the body wall as well as toxicity after gaining entry. Several materials were cited which showed relatively higher toxicity by feeding than by topical application. Toxic insecticides may or may not possess residual effectiveness as the properties accounting for toxicity may be independent of those accounting for chemical stability required for residual effectiveness.

Feeding and topical application tests that can be conducted without seasonal limitation provide a satisfactory basis for selecting materials worthy of further consideration in residue tests or field trials.

With few exceptions the order of effectiveness established by these tests is in general agreement with field results. The marked effectiveness of materials such as dieldrin and Guthion was readily evidenced as was the ineffectiveness of DDT. In considering the usefulness of this procedure, it should be recognized that numerous insecticides selected for comparison are of the same general order of effectiveness and to select between them, as is attempted here, requires a more critical measure of effectiveness than is usually sought in preliminary screening. More likely a new compound of unknown insecticidal activity would be compared with a standard to determine its promise for field testing. While field performance will remain the ultimate test, it becomes increasingly difficult to conduct such tests in the case of the plum curculio. Severe infestations required for thorough field evaluation are virtually nonexistent in commercial plantings, although the species remains a constant threat and requires control measures annually. Under such conditions increased reliance must be placed on other methods of evaluating insecticides.

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Fate of C^{14}-Diethyltoluamide Applied to Guinea Pigs[1]

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Abstract

Diethyltoluamide labeled with C^{14} was applied to the skin of guinea pigs at 6.97–7.11 mg./sq. in. After 6 hours 0.96–0.98 mg./sq. in. had been lost by evaporation and 1.32–3.40 mg./sq. in. by absorption. The remaining repellent was removed. The radioactivity in the urine reached a peak within 12 hours after application, and over 80% of the absorbed dose was excreted in 24 hours; however, diethyltoluamide as such was not found in the urine. Only 0.75% of the absorbed dose was excreted in the feces during 8 days, whereas 93% appeared in the urine. Very small amounts of radioactivity were found in the blood, skin, and hair.

Little information is available as to why certain compounds act as insect repellents, and why they are effective for such short periods of time. In other words, what happens to a repellent after it has been applied to the skin? Answers to this question would go a long way to help in the search for better repellents, a search that has been mainly empirical. An attempt to elucidate the fate of repellents was undertaken at the Orlando, Florida, laboratory through the use of the C^{14}-carboxy-labeled meta isomer[3] of diethyltoluamide (Green 1958), which possesses the highest repellent activity of the three isomers (Gilbert *et al.* 1957). The evaporation and ab-

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[2] Rouselle Sutton, Nelson Smith, and Richard Fye assisted in the experiments.

[3] Used by authorization of the U. S. Atomic Energy Commission.

sorption of this repellent were studied after application to the skin of guinea pigs.

EXPERIMENTAL PROCEDURES.—To study the modes of loss of the repellent each guinea pig was kept in a metabolism cage, which consisted of a glass funnel 30.5 cm. in diameter that contained a 1.3-cm. hardware-cloth screen placed 9 cm. below the top to serve as a platform for the animal. A fine-mesh screen was located in the neck of the funnel to collect the feces, and the urine was allowed to run down the sides of the funnel and collect in a graduated cylinder.

The guinea pig was placed in the cage, and the dorsal surface was treated with C14-diethyltoluamide (12.13 μc./gm.). One-tenth milliliter of a 50% solution of the repellent in ethanol was pipetted onto the shaved area and spread evenly with a glass rod. The repellent adhering to the rod was washed into a volumetric flask with ethanol, and a standard sample consisting of 0.1 ml. of the repellent solution was pipetted directly into another volumetric flask. After the guinea pig was treated, a stainless steel funnel was inverted over the cage and sealed in place with tape. The animal was not restrained on the hardware cloth because the treated area did not touch the sides of the chamber. The steel funnel was connected by a copper tube and rubber connections to two sets of three traps (gas-washing bottles) containing ethanol. The flow of air upward through the cage was maintained by suction at the rate of 26 liters per minute, and the volatilized diethyltoluamide was collected in the traps. Fresh ethanol was placed in the traps when necessary to maintain the desired volume. Air temperature was maintained at 28±1° C.

After 6 hours the guinea pig was removed from the cage and the treated surface was rinsed with 500 ml. of ethanol. The animal was then anesthetized with ether, a sample of blood was taken from the foreleg, and two skin biopsies were made from the treated area. Additional biopsies and blood samples were taken at various intervals. The urine and fecal samples collected throughout the experiment were refrigerated until analyzed for radioactivity.

The amounts of diethyltoluamide in the rinse and traps were determined by ultraviolet analysis (Schmidt *et al.* 1958). The applied dose was calculated by subtracting the amount left on the glass rod from the standard sample, and the repellent absorbed by subtracting that in the rinse and traps from the applied dose.

Preliminary experiments had shown that it was impossible to obtain quantitative oxidation of unlabeled diethyltoluamide by the wet-combustion micromethod of Peters & Gutmann (1953). The following modifications, which insured complete oxidation, yielded quantitative recoveries of C14-diethyltoluamide from samples of urine, blood, feces, and skin: Twice as much potassium iodate and potassium dichromate mixture as recommended by Peters and Gutmann were added to the combustion tube. Both the combustion and receiver tubes were cooled in an ice bath for 5 minutes before the Van Slyke-Folch reagent was slowly poured into the combustion tube. The system was slowly evacuated to a pressure of 20 mm. and isolated from the vacuum pump, the ice baths were removed, and then the reaction mixture was heated gently. After the initial reaction subsided, the mixture was refluxed gently until effervescence ceased and only an occasional bubble arose in the receiver. The 24-hour urine samples and a similar sample from an untreated guinea pig were extracted three times with isooctane, and the ultraviolet spectra of the extracts were examined to ascertain whether any unchanged diethyltoluamide was present.

Table 1.—Evaporation and absorption of diethyltoluamide (mg./sq. in.) on five guinea pigs rinsed 6 hours after application.

Pig[a]	Applied	Evaporated	Recovered in Rinse	Absorbed[b]
		C14-Diethyltoluamide		
A	6.97	0.96	4.69	1.32
B	7.11	.98	2.73	3.40
		Unlabeled diethyltoluamide		
C	7.14	.89	3.21	3.04
D	6.21	1.02	3.98	1.21
E	6.63	.90	3.09	2.62

[a] Area treated, 7.5 sq. in.
[b] Calculated by difference.

RESULTS AND DISCUSSION.—The two guinea pigs that were treated with C14-diethyltoluamide weighed 985 and 740 grams and the dosages were 0.65 and 0.87 μc./gm., respectively. Three other guinea pigs treated with unlabeled repellent served as checks. The amount of repellent applied was approximately 7 mg./sq. in., which is similar to that applied to human skin in practical use. The amounts evaporated, absorbed, and recovered in the rinses 6 hours after application are shown in table 1. The amount absorbed varied greatly from animal to animal, but the rate of evaporation remained almost constant.

The radioactivity, expressed as microgram-equivalents of diethyltoluamide, that was found in the blood, skin, and hair is presented in table 2. The radioactivity disappeared from the skin rather rapidly; for example, in guinea pig B at 198 hours the skin had less than 1% of the radioactivity it contained immediately after it had been rinsed. The alcohol rinse presumably removed the repellent from the surface of the skin, since additional quantities of ethanol gave a negative test; however, radioactivity was detected by a GM tube held 1 inch from the rinsed surface. Immediately after the skin was rinsed, its activity was 3,100 c.p.m. After 72 hours the activity had decreased 50% and after 216 hours it was less than 10% of the 6-hour count. Some of this radioactivity was in the hair growing in the rinsed area. The hair from guinea

Table 2.—The amount of radioactivity, expressed as μg.-equivalents of diethyltoluamide, found in blood, skin, and hair of two guinea pigs at various intervals after topical application.

Hours After Treatment	Per Ml. of Blood		Per Mg. of Skin		Per Mg. of Hair
	Pig A	Pig B	Pig A	Pig B	Pig B
6	2.3	3.0	2.31	2.39	—
24–30	—	1.3	0.76	0.95	—
54	—	—	—	.15	7.5
144–150	—	—	.14	.03	2.9
198	—	—	—	.02	.4

Table 3.—Radioactivity (μg.-equivalents of diethyltoluamide per milliliter) and cumulative per cent of the absorbed dose found in the urine of two guinea pigs treated topically with C^{14}-diethyltoluamide.

Hours After Treatment	Pig A		Pig B	
	Radioactivity	Per Cent Excreted	Radioactivity	Per Cent Excreted
3	6.82	0.61	—	—
4	—	—	7.10	0.13
6½	—	—	563.00	55.31
12	614.21	58.81	—	—
24	130.80	81.66	364.50	83.91
30	32.26	84.07	22.65	86.18
36	25.79	86.51	—	—
48	11.90	89.00	26.50	89.92
54	—	—	18.44	90.50
60	8.04	90.32	—	—
72	7.38	91.20	9.78	92.61
78	—	—	5.86	93.01
96	5.81	91.95	6.14	93.51
120	2.08	92.31	3.66	93.91
144	1.07	92.55	0.82	94.17
168	0.83	92.78	.83	94.40
192	—	—	.57	94.53
216	—	—	.58	94.61

pig B was shaved after 54 hours and analyzed. The hair probably picked up radioactivity from the diethyltoluamide that had penetrated the hair follicles.

The peak of radioactivity in the urine was reached within 12 hours after application. More than 80% of the absorbed dose was excreted in the urine within 24 hours and 89% within 48 hours (table 3). The ultraviolet absorption spectra of the isooctane extracts of urine did not reveal the presence of unchanged diethyltoluamide. This is in accordance with previous unreported findings with the unlabeled repellent applied to human subjects. The aqueous phase was tested for radioactivity after extraction and no decrease in the counting rate was noted, indicating that the diethyltoluamide had been metabolized, probably in the liver, into compounds not soluble in isooctane. The urine was then acidified and extracted with isooctane, and again the activity remained in the aqueous phase. The acidified urine was then continuously extracted with diethyl ether, which removed most of the activity from the aqueous phase. Efforts are now being made to identify the metabolites.

Table 4.—Per cent distribution of the absorbed dose of diethyltoluamide in two guinea pigs.

Material	Pig A (After 168 Hours)	Pig B (After 216 Hours)
Urine	92.78	94.61
Feces	0.74	0.75
Skin	1.38	.03
Hair	—	.56
Total	94.90	95.95

Only small amounts of radioactivy appeared in the feces. The feces of guinea pig B contained a total activity which was about 2.45 times that of guinea pig A; however, guinea pig B absorbed 2.53 times the radioactivity of A (25.48 mg. vs. 9.92 mg.). The radioactivity per gram of feces was much higher in the second animal because the total amount of feces produced was much smaller.

About 95% of the absorbed dose was recovered from each of the treated guinea pigs (table 4). The skin of guinea pig A showed a greater recovery because larger skin biopsies were taken. Much smaller samples were taken from guinea pig B because of improved biopsy techniques; however, when the radioactivity of the skin from the two animals was compared, as is shown in table 2, the results were almost identical.

The over-all recoveries of the applied dose for the two guinea pigs were 99.0% and 98.1%, including the amounts that had evaporated and those found in the ethanol rinses.

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Inheritance of DDT-Dehydrochlorinase in the House Fly[1]

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Abstract

A modification of a method devised by Sternburg *et al.* (1954 Jour. Agric. and Food Chem. 1,125–30) made it possible to assay the DDT-dehydrochlorinase (DDT'ase) in individual flies of a population of DDT-resistant (R) house flies, *Musca domestica* L. By this means it was possible to obtain a measure of the variability of this enzyme in a population. It was also possible to relate the concentration of DDT'ase in parents with that found in the offspring. Regardless of the enzyme activity of the individual parents, that of the progeny appeared to drift toward a mean value.

Flies selected for resistance to DDT alone have considerably less DDT'ase than after being selected for resistance to a combination of DDT and DMC (bis-(*p*-chlorophenyl) methyl carbinol). A strain of flies selected for resistance to combination (DMC-R strain) was used to cross, inbreed and backcross with susceptible flies to determine the inheritance pattern of DDT'ase and its relationship to resistance. By this means a quantitative relationship between presence of DDT'ase and resistance to DDT has been established. Furthermore, the pattern of inheritance of resistance and DDT'ase activity shows no serious discrepancy with the proposal of Milani (1956, Riv. Parassitol., **17**: 223–46), that resistance to DDT may be governed by a single partially dominant gene which behaves according to simple Mendelian principles.

Sternburg *et al.* (1954) have reported that various strains of house flies, *Musca domestica* L. which are resistant (R) to DDT contain an amount of the enzyme DDT-dehydrochlorinase (DDT'ase) corresponding to the level of resistance of the strain to DDT. The development of a method (Lovell 1955) to assay the enzyme in a single R fly suggested that a more precise evaluation could be made of the relationship between these factors. If the enzyme DDT'ase is the principal mechanism causing resistance, it should quantitatively follow the pattern of resistance measured in the progeny of R-parents and crosses, inbreds and back crosses with susceptible (S) flies. The results of such a study might provide additional information concerning the genetic aspects of resistance which now can only be inferred from dosage-mortality data.

Materials and Methods.—As they emerged, the adults of a DDT-R strain of flies were confined to cages treated with 200 gm. of DDT per m². The progeny of the survivors of such exposure were treated in the same manner. This procedure was continued through four generations when it became evident that no significant numbers of flies were dying during the normal life span of the adult because of intoxication from continuous exposure to DDT. The next generation of adults of this strain was used for the assay of DDT'ase concentration in the individuals of a sample of the population. This strain is referred to as the R-strain.

The R-strain was subcultured and succeeding generations of adults were subjected to gradually increasing quantities of equal proportions of DDT and DMC (bis (*p*-chlorophenyl) methyl carbinol) applied to the cages in which the adults were confined. Approximately 90% of the first generation of flies exposed in a cage treated with 2 gm. of the mixture per m.² died before they could reproduce. By gradually increasing the selection pressure on successive generations, approximately 90% of the 17th generation could survive and reproduce in a cage treated with 300 gm. of the mixture per m². This strain was designated as DMC-R strain.

Dosage-mortality data were obtained by the topical treatment of the mesonotum of female flies with graded doses of DDT or 1:1 mixture of DDT and DMC in solution in 95% ethanol or 1:9 mixtures of acetone and ethanol. Ethanol was used for treatments where doses of 20 μg. or less were required and acetone-ethanol for higher doses. The volume of solutions applied varied from 1.3 to 2.6 μl per fly. A group of 25 flies was used for each dosage and the treatments were applied while the flies were under light anesthesia with CO_2. After treatment the flies were transferred to screen-covered pint jars. A sugar solution was provided for food and the mortality was determined 24 hours later.

The method of Sternburg *et al.* (1954) was used to measure the DDT'ase activity of homogenates consisting of large numbers of flies. The actual enzyme concentration in such cases was determined from aliquots of the homogenates which were the equivalent of six flies in a reaction mixture of a final volume of 3 ml. Individual flies were prepared for the assay of DDT'ase by grinding each fly in 1 ml. of glass distilled water for 1 minute at 3° C. in a glass homogenizer. The homogenate was then poured into a vial, stoppered, quickly frozen and stored at −17° C. until ready for enzyme assay.

The method of Sternburg *et al.* (1954) was modified to increase its sensitivity to a point where by trial it was shown that significant enzyme activity could be determined for each individual of a sample of flies taken at random from an R-population. The modification amounted simply to a proportionate reduction in substrate, cofactor and volume of the reaction mixture. Thus 300 mg. of glass beads[3] were placed upon the bottom of a 15 ml., single side arm Warburg flask and covered with 1 ml. of acetone containing 1 mg. of DDT. The acetone was evaporated in an air stream, and 0.5 ml. of 0.137 M phosphate buffer containing 0.009 M glutathione was added to the side arm of the flask. The homogenate of a single fly was then added to the main chamber, the flask attached to a Warburg manometer, and the contents equilibrated to a temperature of 37° C. under an atmosphere of N_2 for 15 minutes. At this time the contents of the side arm were tipped into the homogenate and the reaction was allowed to continue for 2 hours while the vessel was shaken at a rate sufficient to keep the mixture in suspension. The flask was then removed from the manometer and the reaction stopped by the addition

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[3] Minnesota Mining and Manufacturing Co., Minneapolis, Minnesota.

of 1.5 ml. of H_2SO_4. The mouth of the flask was degreased by wiping it with a cleansing tissue moistened with cyclohexane. When the contents of the flask had cooled to room temperature, 8 ml. of cyclohexane was added. The flask was then closed with a ground glass stopper which was lubricated with syrupy phosphoric acid. The flask and its contents were then shaken for 2 hours at the maximum speed of a Burrel wrist-action shaker. After shaking, the cyclohexane layer was allowed to separate and an aliquot was taken from it for the determination of DDT and DDE (2, 2-bis(*p*-chlorophenyl)1,1-dichlorethylene), as described by Sternburg *et al.* (1954).

The relationship of DDT'ase activity in the progeny to that of the parents was tested by the mating of randomly selected pairs from the R strain. A large number of flies were paired, but only a small percentage mated and produced offspring. Pairs which produced progeny were assayed for DDT'ase and compared with their progeny.

Reciprocal crosses made from 100 randomly selected flies of each sex from the DMC-R and S strain provided F_1 generations sufficiently populous to furnish 50 flies of each sex for individual enzyme assay, adequate numbers of females to measure their response to graded doses of DDT, 100 flies of each sex for inbreeding, and the same number of each sex to backcross with the S strain. The F_2 generations and backcrosses to the S strain were carried out in the same manner, and they also provided sufficient numbers of offspring to complete enzyme assays upon 50 flies of each sex and sufficient numbers of females to obtain dosage-mortality data on a representative sample of the generation.

RESULTS AND DISCUSSION.—*DDT'ase in R-strain.* Adults in a random sample of the R-strain, which consisted of 50 flies of each sex, were assayed individually for DDT'ase. All the flies were found to contain a measureable amount of the enzyme. The range of enzyme activity in the sample of the population was found to vary by a factor of about 14. These data, which are plotted as a frequency distribution curve (fig. 1), show that a small percentage of the flies possess an amount of enzyme much in excess of the mean, thus giving a decided skewness to the curve. The departure of the curve from the normal indicated variability beyond that expected from a homogeneous population.

This deviation may be accounted for partially by the fact that approximately 20% of the population are less tolerant than the remainder of the population (fig. 2, R). The distribution also reflects an error of unknown magnitude connected with the preparation and assay of the enzyme in a single fly.

The observed wide variation of DDT'ase activity in individuals of the R-strain raised a question concerning the influence of parental enzyme concentration upon that of the offspring. Randomly selected pairs resulted in the production of progeny from parents having varying levels of DDT'ase activity (table 1). In general the level of enzyme concentration in the offspring appeared to drift toward a mean regardless of the enzyme concentration in the parents. No quantitative difference in the enzyme activity appears in the offspring which can be related to a quantitative difference in one or the other of the parent sexes.

Selection with mixtures of DDT and DMC.—Moorefield

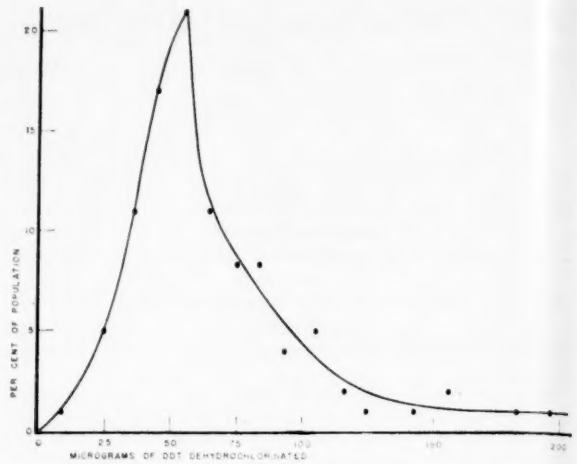

FIG. 1.—The distribution of DDT'ase as obtained from the measurement of the enzyme content in individuals from a randomly selected sample of 100 flies from the DDT-R strain. The sample consisted of equal numbers of each sex, and the incubation period was 2 hours. The respective points represent the average concentration of enzyme for increments of 10 μg. of DDE.

& Kearns (1955) showed that a strain of flies resistant to a combination of DDT and the so-called synergist DMC, possessed considerably more DDT'ase than a DDT-R strain, selected for resistance to DDT alone. Since experiments had been planned for the study of the in-

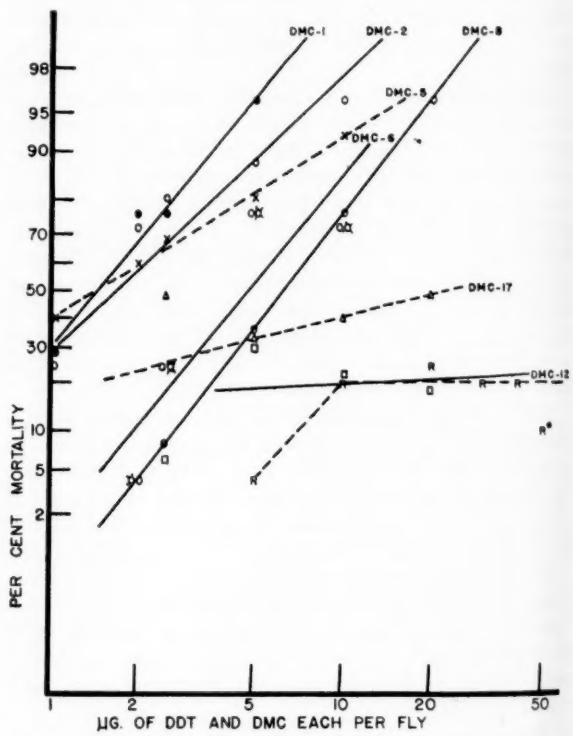

FIG. 2.—Change in dosage-mortality response resulting from the selection of successive generations of the R-strain with a combination of DDT and DMC. The dosage response indicated for the R* strain was obtained from treatment with DDT alone.

Table 1.—DDT-dehydrochlorinase activity in homogenates of each parent and a mass homogenate of their progeny.[a]

μG. OF DDE PRODUCED PER FLY IN 2 HOURS				
Parents				
Male	Female	Average	Progeny	RANGE OF ENZYMATIC ACTIVITY IN PARENTS
14.0	15.0	14.5	43.7	low
11.0	53.0	32.0	44.0	low male, medium female
38.0	71.0	54.5	33.0	low male, high female
37.0	31.0	34.0	43.0	medium
36.0	33.0	34.5	35.4	medium
27.0	47.0	37.0	39.3	medium
44.0	39.0	41.5	54.7	medium
37.0	47.0	42.0	57.3	medium
58.0	48.0	53.0	65.7	medium
51.0	63.0	57.0	43.3	medium
73.0	36.0	54.5	47.7	high male, low female
67.0	83.0	75.0	87.3	high
101.0	94.0	97.5	73.0	high

[a] The parents were randomly selected from the DDT-R strain of house flies.

heritance of DDT'ase, based upon assays of individual flies, it seemed desirable to increase the enzyme concentration in the DDT-R strain by selecting it through a number of generations with a combination of DDT and DMC. It was assumed that if this could be accomplished, it would reduce the possibility of being unable to detect DDT'ase in the progeny of various crosses.

The selection process was carried out through 17 consecutive generations, when it became apparent that 15 to 35% of the population would die from small or maximal dosages of the combination of DDT and DMC. It was indicated that continued selection by this method should not be expected to produce any marked change in the dosage-mortality response (fig. 2, DMC-12, DMC-17).

The log dosage-probit lines drawn in figure 2 are only an approximation of the dosage-mortality response. They represent the best visible fit to data that in general indicate a high degree of heterogeneity in the population. However, the progressive trend toward the development of resistance to the combination of DDT and DMC is evident.

During the selection process, most of the first eight generations and the 17th were assayed for DDT'ase. The enzyme concentration increased progressively as shown in table 2. These findings support the assumption of Moorefield & Kearns (1955) that strains of flies resistant to the combination of synergist and DDT may become tolerant to the combination because of the possession of a greater amount of DDT'ase than a strain selected with DDT alone.

Inheritance of DDT'ase.—Fifty males and fifty females of the DMC-R strain, sampled at random, were assayed individually for DDT'ase. All but two flies proved to have a measurable amount of the enzyme. The average amount of enzyme per fly, in terms of μg. of DDT converted to DDE during a 2-hour incubation period, was 53 and 55 μg. for males and females, respectively. The average of 54 μg. per fly was taken as a value of 100% for comparison with the DDT'ase activity in the progeny of various crosses, as indicated in figure 3.

Table 2.—Increase in DDT'ase activity of the DDT-R strain through successive generations by exposure to cages treated with increasing amounts of DDT and DMC in equal mixture.

STRAIN AND GENERATION	MASS HOMOGENATES μG. DDE PRODUCED PER 6 FLIES PER 2 HOURS
DMC-1	151
DMC-4	183
DMC-6	207
DMC-8	234
DMC-17	277

Reciprocal crosses of DMC-R×S flies produced F_1 generations that were assayed for DDT'ase in the previously described manner. Since no significant difference in DDT'ase appeared, the values obtained were averaged for the two groups and sexes. It will be noted that 96% of all flies had demonstrable quantities of DDT'ase, but the average enzyme activity per fly was approximately 50% of that of the resistant parents. Dosage-mortality data obtained from females of each F_1 generation indicated the absence of flies which could be considered susceptible in terms of the susceptible parents (fig. 4, G, H and S). Since 70 to 80% of the flies could be killed in varying proportions from doses ranging from 2.5 to 50 μg. of DDT per fly, this portion of the population could not be classed as resistant in terms of the R parents, most of which tolerate more than 50 μg. per fly.

It can be concluded that the DMC-R×S crosses result in progeny with approximately one-half the DDT'ase activity of the DMC-R-parents and a degree of resistance considerably less than the DMC-R-parents. The decrease in resistance in the F_1 can only be estimated approximately because it is not possible to apply sufficient DDT to kill the DMC-R-parents.

Both F_2 generations were sufficiently similar in respect to the percentages having DDT'ase and the amount of the enzyme per fly to be averaged. These values are shown in figure 4. In contrast to the DMC-R and F_1 parents, where nearly 100% of the flies could be shown to possess DDT'ase, approximately 35% of this generation lacked a measurable quantity of this enzyme. If the presence of DDT'ase is highly correlated with resistance, then these data would suggest that a segregation of the population into R and S individuals had occurred in this generation. Dosage mortality data (fig. 4, E and F) show that approximately 20% of the population are susceptible to dosages of 1 μg. of DDT per fly or less. Based upon the percentages of flies having DDT'ase, it would be predicted that 35% of the population should be susceptible to DDT. This discrepancy may be significant or just the result of a sampling error. However, limitations imposed by the time consumed in making enzyme assays and the numbers of flies required to complete the whole experiment precluded the possibility of a test designed to clarify this point.

The progeny of four different backcrosses of the F_1 to susceptible flies were assayed for DDT'ase and their response to DDT as shown in figures 3 and 4. The numbers of flies having DDT'ase varied from 50 to 68% and the amount of enzyme contained in the flies varied from 42 to 60% of that contained by the DMC-R parents. The

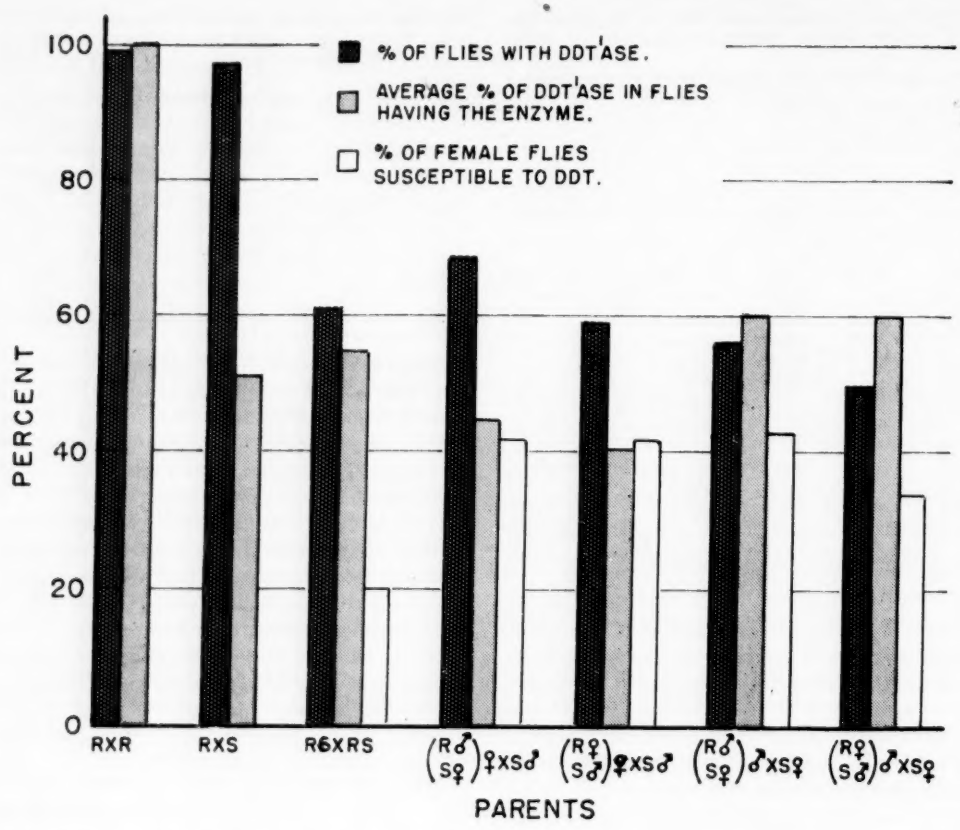

FIG. 3.—The inheritance of DDT'ase and its relationship to resistance as determined from crosses, inbreds and backcrosses of S flies with the DMC-R strain.

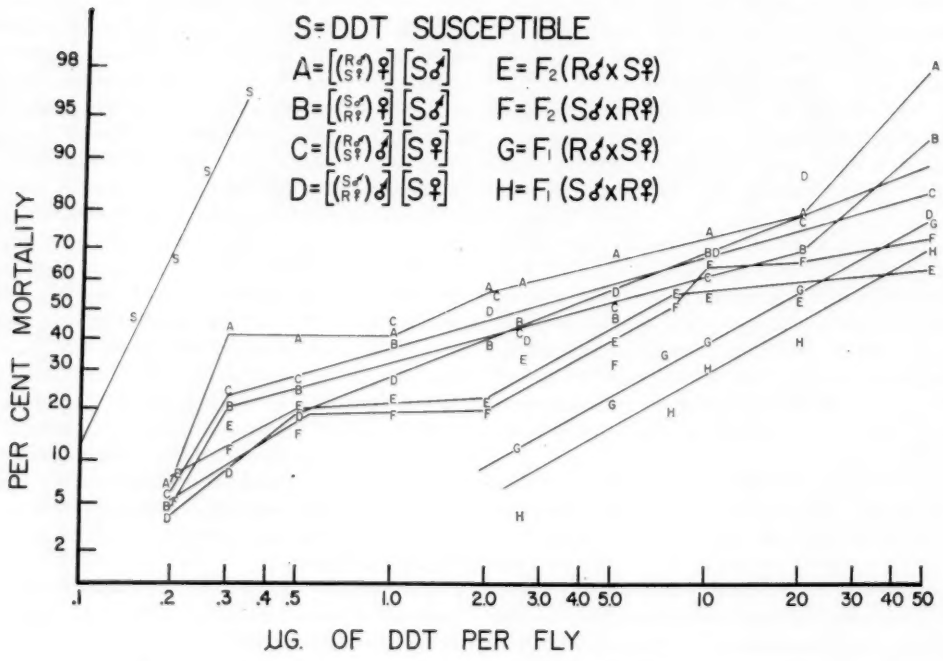

FIG. 4.—Dosage-mortality response of the S-strain and the progeny from crosses of the S-strain and the DMC-R strain.

Table 3.—A comparison of results obtained with those expected assuming the factor for DDT resistance is controlled by a single partially dominant gene which governs DDT'ase activity and follows a simple Mendelian principle.

Assumed Genetic Make-up of Cross	Assumed Ratio and Genetic Make-up of Progeny			Per Cent of Flies with DDT'ase		Per Cent of DDT'ase Relative to the DMC-R Parents		Per Cent of Flies Susceptible to DDT	
	1:2:1			Actual	Theoretical	Actual	Theoretical	Actual	Theoretical
RR×RR	RR			98	100	100	100	0	0
RR×SS	RS			96	100	52	50	4	0
RS×RS	RR	RS	SS	63	75	55	67	20	25
(R♂)♀×SS♂ (S♀)	RS		SS	69	50	45	50	43	50
(R♀)♀×SS♂ (S♂)	RS		SS	58	50	42	50	42	50
(R♀)♂SS♀ (S♂)	RS		SS	55	50	60	50	43	50
(R♀)♂×SS♀ (S♂)	RS		SS	50	50	60	50	34	50

percentage of flies from each group which could be considered susceptible to DDT varied from 34 to 44%. These values are close to those expected if the susceptible flies are considered to be represented by that fraction of the population having no measurable amount of DDT'ase and being susceptible to a dosage of 1 μg. or less of DDT per fly. There is no question but that a clear-cut segregation occurred, which resulted in flies having DDT'ase, lacking DDT'ase, and flies resistant or susceptible to DDT.

Milani (1956) has proposed that DDT-resistance in the house fly may be controlled by a single gene, which is partially dominant for the characteristic. It seemed desirable, therefore, to test our data upon enzyme activity and susceptibility to DDT in the framework of this simple Mendelian principle. Experimentally it has been shown that essentially all of the individuals of the DMC-R strain were resistant to DDT and contained a measurable amount of DDT'ase. Each individual fly in the F_1 generation from crosses between DMC-R and S flies also had measurable quantities of DDT'ase, amounting as an average to one-half that of the DMC-R parents. They were also resistant to DDT, but at a lower order than that of the DMC-R parents. Based upon these facts, actual findings can be compared with those expected from a genetic relationship as proposed by Milani (table 3).

That a clear genetic relationship exists between DDT'ase and resistance is evident. Furthermore, the data show no serious discrepancy from the pattern of inheritance of resistance as proposed by Milani. It is not intended that these results should be interpreted as evidence for the pattern of inheritance of resistance for all DDT-R strains.

Lichtwardt *et al.* (1955) refer to an inbred resistant strain (IR-1) having a single autosomal dominant gene for high tolerance to DDT. Sternburg (1955), however, assayed the homozygous parents of this strain and the F_1 from a cross with an S-strain for DDT-ase and found the F_1 generation to have approximately one-half the amount of enzyme determined for the resistant parents. It seems possible that intermediate levels of resistance may exist as heterozygotes, but are difficult, or perhaps in some cases impossible, to distinguish, in terms of dosage response, from parents homozygous for resistance.

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Spider Mites on Walnut in Northern California[1]

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ABSTRACT

The problem of spider mites on walnut has become more acute since the advent of DDT and other newer organic insecticides. With DDT the increase in the spider mite population mounts as the dosage is increased.

The principal species of spider mites involved are the Pacific spider mite (*Tetranychus pacificus* McG.), two-spotted spider mite (*T. telarius* (L.)), and the European red mite, *Panonychus ulmi* (Koch).

Induced increases in spider mites involve other factors in addition to interference of insecticides with natural enemies. These factors may exert their influence either upon the spider mite or through the host.

Populations of the Pacific spider mite and the two-spotted spider mite, but not the European red mite, have been suppressed where schradan has been used to control the walnut aphid. With the European red mite schradan induces an increase in the population. In the hot interior San Joaquin Valley, which has a climate less favorable for European red mite than the more coastal regions, natural enemies usually control the pest before it has had opportunity to do serious damage. In the cooler regions natural enemies may fail to be effective and acaracides may be required.

Walnut can tolerate a relatively large spider mite population. This tolerance allows sufficient time to determine whether natural enemies will have an opportunity to control the pest. Natural enemies frequently reduce the spider mite to a subeconomic level even though spider-mite-inducing materials such as DDT or Sevin® (1-naphthyl-*N*-methyl carbamate) are used.

Control of spider mites has been complicated by the problem of resistance. A more effective acaricide may become a spider-mite-inducing agent once the pest develops resistance to it. Of the newer acaricides tested the most effective were Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) and Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol).

Spider mites have long been known as a pest of English or Persian walnut. DeOng (1918) reported their seriously attacking this crop. Since then numerous papers have appeared reporting injury by spider mites. General and widespread attacks were apparently uncommon, and as late as 1945 Batchelor *et al.* (1945) stated that spider mites are sporadic in occurrence but usually not a widespread pest of walnut.

POSSIBLE CAUSES OF SPIDER MITE INCREASE.—The mite problem has become increasingly acute with the advent of DDT and related insecticides (Michelbacher *et al.* 1946). Michelbacher & Middlekauff (1949) observed that the seriousness of the problem mounted as the amount of DDT used to control the codling moth, (*Carpocapsa pomonella* (L.)) was increased. For this reason low dosages of DDT were recommended (Michelbacher & Bacon 1952). The threat of a serious increase in the spider mite population was greatly reduced but not entirely eliminated when the actual amount of DDT per 100 gallons of spray was 0.5 pound. Many factors other than insecticides may be responsible for an increase in the spider mite population, and Middlekauff & Michelbacher (1951) noted that when natural conditions favor the spider mite, heavy populations may develop in orchards that have received no application of insecticides of any kind. In such situations it is difficult to determine what part, if any, insecticides may have played in increasing the spider mite population. Although this population increase in untreated orchards sometimes occurs, abundant evidence exists that links certain treatments with induced increase in the spider mite population (Fleschner 1959). In some cases the increase may be due to interference with natural enemies, but other factors are also involved. As an example, Davis (1952) observed that DDT may cause a dispersion of mites, resulting in a higher reproductive potential and hence a more rapid increase in the population. Fleschner (1952), who conducted investigations on the citrus red mite, (*Panonychus citri* (McG.)) reported indications that deposits of field dust and DDT spray materials lowered the host-plant resistance to mite attack through certain physical properties of these materials. Further, he noted that DDT sprays apparently produced some change within citrus leaves that also resulted in a lowering of the host-plant resistance sometime after the application was made. Increase in spider mite damage has often been associated with dryness and dusty conditions. McGregor (1949) reported that most species of spider mites thrive under relatively high temperatures and a minimum of rainfall. He also noted that the heaviest infestations occurred normally during July and August.

In the case of walnut, insecticides other than DDT have been responsible for induced increase in the spider mite population. In this regard, the action of schradan has been most interesting. Michelbacher & Oatman (1955) reported the material was effective against the Pacific spider mite (*Tetranychus pacificus* McG.) but not the European red mite (*Panonychus ulmi* (Koch)). Later Michelbacher & Hitchcock (1958) stated that schradan gave effective suppression of the Pacific spider mite and the two-spotted spider mite (*Tetranychus telarius* (L.)) but not the European red mite, at all dosages that were effective against the walnut aphid, *Chromaphis juglandicola* (Kalt.). Where the dosage of schradan approached 3 pounds or more per acre, however, it tended to hold the European red mite in check (Michelbacher 1956b). The tendency of schradan to induce an increase in European red mite population has been observed wherever it has been applied on walnuts in northern California, but the problem resulting from its use has shown considerable variation in the different areas where it has been applied. At Linden, which is located in the hot San Joaquin Valley, the population of the European red mite has not reached a destructive level following treatments of schradan. In

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this area natural enemies tend to suppress the mite population before actual damage occurs. At San Jose, which is located at the south end of San Francisco Bay and has a cooler climate, the situation is different from that encountered at Linden. Here, in spite of considerable action by natural enemies, the European red mite population usually increases to a very high level where schradan is applied. Often it is necessary to apply a treatment to control the mite. At Walnut Creek, which has a climate similar to San Jose, the European red mite population tends to increase to a high l evel where schradan is used, but in most cases it has been reduced to a nondestructive level by an increase in natural enemies.

The difference encountered in the destructiveness of the European red mite in northern California following applications of schradan may be associated with climate. Ortega (1953), who conducted investigations in southern California, reported that in the warmer interior areas the two-spotted spider mite and closely related species supplant the European red mite as the primary mite pest of walnut. Therefore, under the hot conditions of the San Joaquin Valley it is possible that the European red mite may not thrive to the same extent as it does in the cooler regions, and as a result it is more easily subjected to control by natural enemies. However, it should be pointed out that schradan treatments have been responsible for the development of destructive populations of the European red mite in the interior Sacramento Valley, which has a climate similar to that found in the San Joaquin Valley.

NATURAL ENEMY—HOST RELATIONSHIPS.—Walnut is fairly tolerant to spider mites. A rather large population is required before damage of economic proportions results. This is a fortunate situation, for it allows sufficient time to determine adequately whether or not natural enemies will effectively control the pest. Although no exact studies have been made, the natural enemy-host relationship has been followed on many occasions. These researches have demonstrated the importance of such enemies in checking damage by spider mites. Often a rapid increase in the spider mite population has been followed closely by a sufficient increase in natural enemies to control the pest. In some cases natural enemies failed to accomplish this and in others they secured control just prior to the appearance of economic damage. In some instances several weeks elapsed before the outcome could be definitely determined. Of the natural enemies, lady bird beetles of the genus *Stethorus* appear to be the most important. Other effective predators are mites of the genus *Typhlodromus*, anthocorid bugs, the green lacewing, and the six-spotted thrips, *Scolothrips sexmaculatus* Perg.

Because of the rapidity with which spider mites develop resistance to acaricides, the beneficial actions of natural enemies should be utilized to the fullest extent. Frequent and unnecessary treatments add to the problem. Difficulties increase as the pest develops resistance to more and more materials. In part, and with some materials, this resistance results from the once effective acaricides' actually acting as a mite-inducing agent. For example, Ortega (1956) reported demeton as giving excellent control of spider mites on walnut in southern California and similar results were experienced by Michelbacher (1956b) in northern California. Later, however, Michelbacher & Burdick (1958) not only observed the European red mite showing resistance to demeton in the San Jose region, but noted that the problem was increased where that insecticide was used.

TREATMENTS AGAINST PRINCIPAL SPIDER MITES.—The three principal species of spider mites that are troublesome in northern California are the Pacific mite, the two-spotted spider mite, and the European red mite. The most troublesome species during the past 2 years has been the European red mite. This species has been of particular concern in the experimental orchard at San Jose, an area where resistance is becoming an increasing problem. A serious spider mite population can be expected each year, and this infestation is most likely to occur if a mite-inducing chemical is used in the walnut pest control program. Because of the potential mite problem, most investigations with acaricides for the past few years have been conducted in this San Jose orchard.

The experimental treatments have been applied with an air-carrier sprayer in approximately 200 gallons of water per acre. For population determination the next-to-terminal leaflets were gathered at random from areas that showed evidence of mite feeding. On each leaflet the active stages were counted on both the upper and lower surface in three equally spaced marked zones along the midrib. The count areas were made with a beveled-edge tube having an inside diameter of 15 mm.

In 1958 a serious European red mite infestation was expected, and for this reason the acaricides Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) and Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) were incorporated with the Sevin® (1-naphthyl-*N*-methyl carbamate) treatments applied in May against the codling moth. To serve as a check the acaricide was eliminated from one Sevin treatment. Other materials tested included schradan, ethion, Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate). The first three were applied in combination with DDT, which was used to control the codling moth. The treatments, dates applied, together with the number of European red mites per leaf sample are given in table 1.

Excellent control of the spider mite for the entire season was obtained where either Tedion or Kelthane was incorporated with the Sevin spray applied on May 16. These findings substantiate the results obtained in the preceding season, where these two acaricides were incorporated with a May DDT spray. By August 1, the Sevin plot in which no acaricide was incorporated with the May spray became so heavily infested that it was treated with Tedion to control the mite. At time of this treatment, severe damage and some defoliation were occurring. The injury was sufficiently great to lower materially the quality of the harvested crop. A crack test at harvest showed that 43% of the nuts were shriveled, as compared with 23% in the plots that had received acaricides.

As expected, schradan demonstrated its tendency to induce an increase in the European red mite population. The mite population multiplied rapidly, and although a large predator population developed, a Tedion treatment became necessary on July 25. Despite the fact that mite

Table 1.—Control of the European red mite on English walnuts at San Jose, California.[a]

Treatment and Pounds of Insecticide[b] Applied per Acre	Dates of Application	Average Number of Mites per Leaf Sample[c] on: June 19	June 26	July 11	July 18	July 25	Aug. 1	Aug. 15	Aug. 27	Sept. 19
(Check)										
Sevin 50% 8 Tedion 50% 6	May 16 and July 1 August 1	0.23	0.59	4.19	2.73	19.14	11.68	0.09	0.01	0.00
Sevin 50% 8+Tedion 50% 6 Sevin 50% 8	May 16 July 1	0	0	0.01	0	0	0	0	0	0.03
Sevin 50% 8+Kelthane 18.5% 12 Sevin 50% 8	May 16 July 1	0	0	0	0	0.20	0.01	0.08	0.33	0.23
DDT 50% 8+schradan (4 lb. per gal.) 1.1 Tedion 50% 5	May 16 July 26	1.14	3.50	10.85	6.67	11.25	1.96	0.17	0	0
DDT 50% 8+ethion 25% 8 Ethion 25% 8	May 16 July 18	0	0	0	0	0	0.03	0	0	0
DDT 50% 8+Trithion (4 lb. per gal.) 1 Trithion (4 lb. per gal.) 1	May 16 July 18	0.67	0.64	1.28	0.75	2.63	1.68	2.21	1.42	0.51
Guthion 25% 6	May 16 and July 1	0	0	0.01	0	0	0.01	0.07	0.16	0.72

[a] Treatments applied with an air-carrier sprayer in approximately 200 gallons of water per acre.
[b] Wettable powder unless otherwise shown.
[c] Area of leaf samples 15 mm. in diameter.

injury was plainly visible, the best quality of walnut was secured from this treatment. This fact probably resulted from the outstanding control of the walnut aphid obtained with schradan together with the fact that this systemic insecticide exerts a stimulating action in addition to insect control (Michelbacher & Hitchcock, 1958).

Two applications of ethion and Guthion gave very good control of the European red mite. The results with ethion substantiate the findings of the previous season. The control obtained with Trithion was somewhat disappointing. In places injury by the European red mite was very noticeable. This condition is in contrast to results secured in previous years, and probably indicates that the European red mite is developing resistance to this material. At the time of the second Trithion treatment a large population of predatory mites of the genus *Typhlodromus* was all but eliminated by the treatment.

A threatening European red mite population developed in the schradan plot at Walnut Creek. This condition was paralleled by an increase in the ladybird beetle, *Stethorus picipes* Casey, and a species of *Typhlodromus*. However, by August 8 it appeared that natural enemies might not suppress the pest before too much damage was done. As a precaution the plot was treated with Kelthane (1 gallon 18.5% emulsion in 200 gallons of water per acre) on that date. The treatment almost eliminated the mite from the plot. Average mite counts per 15-mm. leaf samples before and after treatment were: August 5, 10.09; August 8, 9.07; August 15, 0.00; and September 16, 0.00.

Under ordinary conditions the European red mite does not spin any considerable amount of webbing. However, on numerous occasions where high populations existed, many individuals have been observed to be suspended by webbing from the trees. Sometimes they hang from the trees in festoons. This reaction must be a means for them to spread by wind, and one that may alter the population on the trees to a considerable extent. In this connection it is interesting to note that Fleschner *et al.* (1956) observed a mass wind-borne migration of the citrus red mite from a heavily infested to an uninfested citrus grove. In the infested grove, the mites spun down from the leaves by the thousands and were wind carried to the adjacent orchard.

Possible Effectiveness of Natural Enemies as Control Factor—The investigations on walnuts have shown that there is an excellent chance that natural enemies will effectively control spider mites if treatments directed against other pests of walnut are held to a minimum. Often this control is accomplished even though mite-inducing materials such as DDT and Sevin are used. It is important, however, that these materials be thoroughly applied and used at the lowest possible concentration that will insure effective control of the pest against which they are directed. Frequent and unnecessary treatments can prove to be very troublesome. While a considerable mite population can be tolerated, treatment for mite control should be applied as soon as it becomes apparent that natural factors cannot cope with the situation.

It is of interest to contrast the situation of spider mites on walnut with that existing in a susceptible crop, such as pear. A spider mite population that would cause considerable injury to pear would in all probability have little influence on walnut. The fact that pear cannot tolerate a sizeable spider mite population may be responsible in part for the statement by Huffaker & Spitzer (1950) that their observations failed to show any correlations between the European red mite population and the natural enemies present, indicative of a major controlling influence of the latter over the red mite. It is possible

that on pear, control measures must be applied before the beneficial action of the natural enemies have had an opportunity fully to exert themselves.

Although not a true spider mite, the citrus flat mite (*Brevipalpus lewisi* McG.) deserves mention. This mite was first reported on walnut by Michelbacher (1956a). A localized infestation was first noted in the experimental orchard at Linden, California, in 1953. Since then it has been found widespread throughout the Linden and adjacent areas. The citrus flat mite usually does not develop to a destructive population until September. Mention of this pest is important in this discussion because the damage it inflicts tends to mask that caused by the true spider mites. Additionally, the natural enemies that are so important in controlling the true spider mites apparently have little effect upon the citrus flat mite. Because of their small size citrus flat mites are not easily seen by growers, and damage is likely to be attributed to the Pacific spider mite or the two-spotted spider mite. The damage to walnut by the citrus flat mite is similar to that caused by the true spider mites except that no webbing is associated with the injury. Some treatments used in the walnut pest-control program tend to suppress the citrus flat mite while others stimululate an increase in the population. A further account of this potential pest will be given in a separate paper.

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Observations in Wyoming on the Alfalfa Weevil and Its Control

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ABSTRACT

The alfalfa weevil (*Hypera postica* (Gyll.)) is not uniformly distributed throughout Wyoming. The uninfested areas are cooler and have a much shorter growing season than the infested areas. Precipitation seemingly plays little part. The distribution of larvae in a single field was found to be uniform, while the adults tended to be more numerous near the edge of the field, where suitable hibernating quarters were available.

Granulated heptachlor gave better control of weevil larvae than granulated dieldrin. However, dieldrin granules applied at 1.25 lbs. per acre gave good control for 2 years. Untreated plots had more plant bugs (*Lygus* spp.) than plots treated with granulated dieldrin. More pea aphids (*Macrosiphum pisi* (Harr.)) were in the treated plots than in the untreated check plots. In other tests initial control of larvae was achieved with malathion and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl-dimethyl phosphate, 60% technical) spray. Later demeton and Phosdrin-treated plots had the lowest larval population.

Alfalfa fields that had never been treated with dieldrin for the control of alfalfa weevil had three to seven times as many adult weevils in the spring as fields that had been treated at least once in 3 years.

Of the insects that attack the alfalfa hay crop in Wyoming, the alfalfa weevil (*Hypera postica* (Gyll.)) is

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usually the most destructive. Yet in 1955–57 the alfalfa weevil, because of low populations, caused little damage to alfalfa. In 1958 it again became conspicuous and was of economic importance. Observations on weevil control and behavior were made in 1955–58 and are here recorded.

DISTRIBUTION STUDIES.—During the 35 years or more that the alfalfa weevil has existed in Wyoming, it has failed to infest all parts of the State. Sweetman (1929, 1932) and Sweetman & Wedemeyer (1933) have given a detailed report on the ecology of the weevil in Wyoming. Hamlin *et al.* (1949) have summarized the effects of weather on the alfalfa weevil in other parts of the West.

To obtain an idea of why the weevil has not spread over the entire State, weather data (Disterdick 1941) from 5 uninfested alfalfa-growing areas were compared with data from 10 infested areas (table 1). Since there is very little difference in rainfall between infested and uninfested areas, precipitation seemingly plays little part in the establishment of the weevil in Wyoming. The uninfested areas were found to be cooler and have a shorter growing season than those areas having the weevil. In 1956 alfalfa weevil adults and larvae were found in normally uninfested alfalfa fields near Laramie. This infestation failed to establish itself, as no evidence of weevil was found in 1957 or 1958.

Table 1.—Average weather conditions in 4 areas of Wyoming uninfested with the alfalfa weevil and in 10 infested areas.

CATEGORY	TEMPERATURE (° F.) Average Annual	January	July	GROWING SEASON (DAYS)	ANNUAL PRECIPITATION (INCHES)	NO. YEARS WEATHER OBSERVATIONS MADE
Uninfested	43.2	18.10	63.30	90.5	13.90	26–40
Infested	45.4	20.46	70.22	126.8	13.17	18–40

The distribution of alfalfa weevil larvae and adults was studied in a 3-acre field at Torrington. The field was divided on July 23, 1958, into 18 plots of equal size, and 10 sweeps were taken in each plot and the larvae and adults counted. As shown in figures 1 and 2, the weevil larvae were distributed rather evenly over the field, while the adults were more numerous near the south end of the field, where there was a stand of large cottonwood trees. Hamlin *et al.* (1949) noted that adult weevils tended to assemble along ditches, edge rows, et cetera, where they hibernated. Other insects, plant bugs (*Lygus* spp.), pea aphids (*Macrosiphum pisi* (Harr.), lady beetles (Coccinellidae) and damsel bugs (Nabidae)) in the Torrington field followed neither the adult nor the larval weevil pattern.

CONTROL WITH GRANULATED INSECTICIDES.—In 1956 a field near Lingle was divided into plots 18′ by 125′ for a study of the effectiveness of granulated dieldrin and granulated heptachlor for weevil control. Each treatment and an untreated check were replicated five times. Materials used were 2% 30/60 mesh dieldrin and 2.5% 30/60 mesh heptachlor. Each was applied April 16 so as to give 4 ounces of actual material per acre. On May 31, 50 sweeps were taken in each plot and adult and larval weevils counted. The dieldrin plots contained 86 larvae per 250 sweeps, the heptachlor plots 52, and the untreated checks 126. Although the population of weevil larvae was low, the results approximate those of Muka (1957) and Blackburn (1957), who found that granulated heptachlor gave better control of alfalfa weevil than granulated dieldrin. Walstrom & Lofgren (1957) found that dieldrin granules gave effective weevil control in South Dakota. MacCreary (1957) reported dieldrin spray more effective than heptachlor spray in Delaware.

Because of the long residual property of dieldrin, the possibility of its providing control for more than 1 year was investigated. On May 23, 1957, a 5% granulated

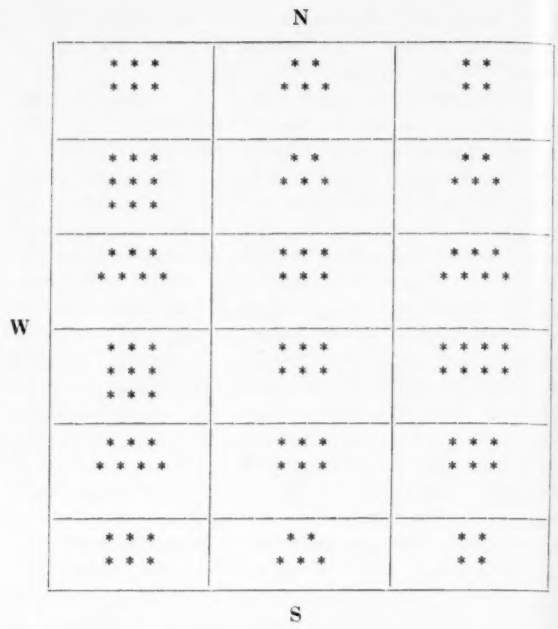

FIG. 1.—The distribution of alfalfa weevil larvae in a field at Torrington, Wyoming, 1958. Each asterisk represents 2 larvae in 10 sweeps of an insect net.

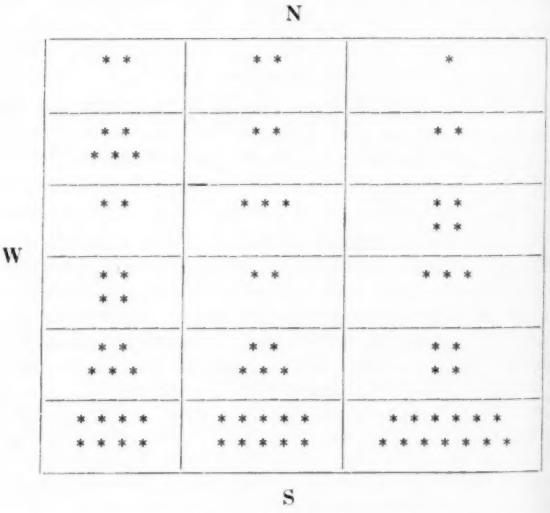

FIG. 2.—distribution of alfalfa weevil adults in a field at Torrington, Wyoming, 1958. Each asterisk represents 1 weevil adult in 10 sweeps of an insect net.

Table 2.—Insect population in 20 sweeps of an insect net in alfalfa plots treated with 1.25 lbs. dieldrin and in untreated check plots. Torrington, Wyoming, 1958.

Insect	Treated Plots		Untreated Plots	
	June 5	July 9	June 5	July 9
Alfalfa weevil:				
adults	7	0	16	3
larvae	16	9	1,741	13
Plant bugs	6	8	30	4
Pea aphids	86	301	22	97
Lady beetles	3	34	2	7
Damsel bugs	0	7	6	5

dieldrin was applied at the rate of 1.25 lb. of actual insecticide per acre to 2 plots, each 77′ by 144′, at Torrington. Duplicate untreated plots of the same size were left as checks. On June 10, July 3, and July 17, 10 sweeps were taken in each plot and the weevil infestation sampled. On June 10 4 larvae per 20 sweeps were found in the treated plots and 120 larvae in the checks, which indicated that control was achieved with this late application.

In 1958 these same plots were sampled on June 5 and July 9. Counts were made of alfalfa weevil, plant bugs, pea aphid, lady beetles, and damsel bugs. The results are shown in table 2. The weevil population was high in 1958 around Torrington, yet the dieldrin-treated plots remained practically free from damage. The alfalfa on the treated plots was several inches taller in June and was conspicuous in the surrounding damaged alfalfa. By July 9 most of the larvae and adults had disappeared from the test plots.

Other Alfalfa Insects.—An indirect effect of weevil control appeared to be reflected in the population of other alfalfa insects. The drying out of infested alfalfa made it more attractive to plant bugs, and more of these insects were found in the check plots than in the treated plots. The plant bug population evened out in the growth of the second cutting. On the other hand, pea aphids increased on moist, succulent growth, and many more aphids were found in the treated plots than in the check plots. On July 9 the pea aphid population was still much higher in the dieldrin-treated plots. Lady beetles had not yet moved into these plots by June 5, but by July 9 they were numerous in the plots having the most aphids. Damsel bugs were more numerous in the check plots on June 5, probably because of the high plant bug population, but by July 9 were evenly spread through all plots.

An estimate of the yield of hay was taken by cutting the alfalfa from a square yard from each plot and weighing it immediately (table 3). A greater difference appeared between yields of the first cutting than of the other two. Because weevils do most of their damage early, the effects of control would naturally be reflected in this first cutting.

Table 3.—Yield of green alfalfa from 2 square yards from plots treated with 1.25 dieldrin and from untreated check plots. Torrington, Wyoming, 1958.

Category	Pounds of Green Alfalfa on Dates Shown			Total (Lb.)
	June 10	July 22	Sept. 10	
Treated	10.8	11.4	10.7	32.9
Untreated	8.5	10.9	9.1	28.5

Table 4.—Populations of the alfalfa weevil in 50 sweeps of an insect net in alfalfa plots. Torrington, Wyoming.

Treatment and Dosage per Acre	July 18, 1957		July 10, 1958		July 22, 1958	
	Adults	Larvae	Adults	Larvae	Adults	Larvae
DDT, 1.5 lb.	10	8	6	8	3	11
Heptachlor, 4 oz.	0	4	9	17	1	35
Demeton, 3 oz.	2	9	12	9	1	5
Phosdrin, 4 oz.	2	7	5	5	2	1
Endrin, 4 oz.	2	1	12	13	2	32
Toxaphene, 2 lb.	2	2	7	9	0	50
Dieldrin, 4 oz.	1	0	5	17	2	21
Parathion, 3 oz.	0	3	3	5	0	22
Malathion, 1 lb.	0	0	2	5	4	28
Untreated	5	23	21	18	5	62

On July 16, 1957, and on July 9, 1958, at Torrington, plots 30′ by 22′ replicated five times were sprayed with the chemicals as listed in table 4. These plots were being used to study control of sucking insects, and observations on alfalfa weevil were secondary. A border 10 feet wide was cut around each plot before spraying and was kept mowed for the remainder of the year. The chemicals were applied by a power sprayer with a 20-foot boom mounted on a truck. The pressure was 75 p.s.i., and all chemicals were applied in 12 gallons of spray per acre. The weevil population was estimated by taking 10 sweeps in each plot on July 16, 1957, and on July 10 and 22 in 1958. No statistical analysis of these data was attempted since the larvae were rapidly disappearing from the field because of pupation and other factors, and the differences noted might be misleading. The results shown in table 4 can, however, be used as indications for further study.

In 1957, a year of light weevil populations, the plots sprayed with malathion, dieldrin, and endrin showed the least infestation of weevil larvae. Endrin was not so effective in 1958 as it was in 1957. Others, too, have found that endrin gave poor weevil control (MacCreary 1957). The best initial control of the larvae in 1958 was shown by malathion and Phosdrin®, (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) however, 13 days after treatment Phosdrin and demeton appeared to be the best. Of the chlorinated hydrocarbons, DDT seemed to give the best larval control. One day after treatment, adult populations were lowest in plots treated with Phosdrin, parathion, and malathion. Thir-

Table 5.—Reinfestation by the alfalfa weevil of alfalfa fields following spraying with dieldrin. Torrington, Wyoming.

Age of Stand (Yrs.)	1952	1953	1954	1955	Average No. Adults[b]
2			×[a]		1
2					7
3					4
3		×	×		1
4					7
4	×				2
4		×	×		1

[a] ×=years in which fields were dieldrin-treated in spring.
[b] In 200 sweeps in the spring of 1955.

teen days later the number of adults remaining were so few that it was difficult to measure the effects of the chemicals.

RATE OF REINFESTATION.—The rate at which alfalfa weevil reinfest alfalfa fields following treatment as outlined by Hastings & Pepper (1951) was investigated in 1955. Five fields for each category shown in table 5 were located in the area around Torrington. Two hundred sweeps were taken in each field in May and the adult weevils counted. The infestation was light in 1955, just as it had been the preceding year, and few farmers sprayed.

Where the fields had never been sprayed, the average number of adult weevil in 200 sweeps was three to seven times as great as where treatment had been applied at least once in 3 years. This result seems to indicate a slight amount of migration into treated fields during years of low population densities. Most of the growers in this area believe they can treat one year and then omit treatments a year without experiencing an economic loss. The experience gained in 1955 would seem to make this plausible at least in years of relatively low populations.

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Effects of Number of Test Insects, Exposure Period, and Posttreatment Interval on Reliability of Fumigant Bioassays[1]

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ABSTRACT

Studies were designed to determine the reproducibility of results from standardized laboratory fumigation tests using an 80:20 (CCl_4:CS_2 by vol.) fumigant in empty 20-liter bottles at 80° F. The test insects were adults of rice weevil (*Sitophilus oryza* (L.)), confused flour beetle (*Tribolium confusum* Duv.), and saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)) and three age groups of immature rice weevils.

Analysis and evaluations showed that (1) the optimum number of adult insects used in each test cage was 50; (2) rice weevil and confused flour beetle should be held at least 10 days and saw-toothed grain beetle 15 days after fumigation for final mortality counts; (3) the descending order of resistance to the fumigant was rice weevil pupae, eggs, and larvae, and adult saw-toothed grain beetles, confused flour beetles, and rice weevils, and (4) variation in results was unpredictable although variations were greatest with rice weevil adults and smallest with rice weevil larvae. Results of parallel experiments conducted on different days were highly variable in some instances, but replicates within a given day's tests were reasonably consistent. Adult insects demonstrated greater variation following longer exposures to the fumigant than following shorter exposures. Immature weevils responded erratically following the shorter exposures but varied least of all groups in the 48-hour exposures. Sublethal treatments of immature rice weevils retarded their rate of development. LC values with their respective confidence intervals were determined. The observed LC_{50} and LC_{90} values associated with 24-hour exposures were 290 and 370 μM./l. (micromoles per liter) for confused flour beetle adults, 165 and 220 μM./l. for rice weevil adults, 125 and 500 μM./l. for rice weevil eggs and young larvae, 155 and 345 μM./l. for young and middle-aged rice weevil larvae and 375 and 865 μM./l. for older rice weevil larvae and pupae. LC values for 4- and 8-hour exposures were greater, and those for 48-hour exposures were smaller, than the 24-hour LC values.

The reliability of experimental results is of major concern since much effort and money are spent in conducting laboratory and field tests with fumigants on stored-product insects. Reliability is dependent, to a large extent, on understanding the factors contributing to variations and the means of keeping these factors more nearly constant. Although some variation in toxicological results is generally expected, it often is great. Gunther *et al.*

[1] Contribution No. 733, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. The financial costs of these studies were supported partially by the Diamond Alkali Company. Investigations were conducted in the Stored-Grain Insects Laboratory, U. S. Department of Agriculture, under the direction of D. A. Wilbur, Department of Entomology, Kansas State University. The valuable assistance of Orlo K. Jantz, Entomologist, U. S. Department of Agriculture, is acknowledged. Accepted for publication April 13, 1959.

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(1958) found that the average of a series repeated at different times varied as much as 600% in LT_{50} values and 1,000% in LD_{50} values. Their tests were with diphenylmethane derivatives and *Tribolium confusum* Duv.

Sun (1947) provided an important contribution in the identification of factors affecting fumigation results. He pointed out some of the biological variations and experimental errors that contribute to highly variable results from parallel tests. He further stated that the inherent individual differences among various testing conditions should be evaluated by each investigator and should be limited by a special experimental design to fit the case.

Many questions remain unanswered and the present study was an effort to elucidate some of the variable factors involved in such research. The major objective was to study variations in results of standardized laboratory fumigations. Specific tests were made to (1) determine the optimum number of adult insects to use per cage; (2) establish the optimum time intervals at which postfumigation mortality counts should be taken; and (3) evaluate the relative toxicity of 80:20 (CCl_4:CS_2 by vol.) to different test insects for different exposure periods. The first two phenomena fundamentally affect the efficiency and reliability of research. The selection of 80:20 as a fumigant was made on the basis of its wide use and composition of two different toxicants.

MATERIALS AND METHODS.—Fumigations were performed in 20-liter glass bottles (fig. 1) in a manner slightly modified from that described by Dennis & Whitney (1954). Each bottle was fitted with a one-hole neoprene stopper holding a pyrex stopcock. A folded strip of blotter paper was attached to the lower end of each stopcock to catch and hold the liquid fumigant as it was being introduced into the fumatorium. Cylindrical test cages, 2½ inches long by ½ inch diameter made of 60-mesh brass screen, were used to hold the test insects. Each cage was closed with a waxed cork to which was attached a waxed string. The test cages were suspended in the center of each bottle by taping the waxed strings to the outside of each fumatorium by an identification tag made of masking tape. A vacuum of 15 inches of mercury was drawn in each fumatorium immediately before fumigating to aid in the introduction, vaporization, and dispersion of the fumigant. A graduated pipet was used to measure and introduce the liquid fumigant into the bottle via the stopcock as the vacuum was being satisfied. Gas analyses were made at the end of each fumigation period and the test insects were removed for mortality counts.

Fumigant.—Standard 80:20 (CCl_4:CS_2 by vol.) was formulated in the laboratory at room temperature (75° F.) from technical grade materials. This mixture was stored in a tightly sealed bottle at 40° F. but was warmed to room temperature just prior to introducing the fumigant into the fumatoria.

Fumigation Conditions.—The fumigations were conducted at prevailing atmospheric pressures at a temperature of 80° F.

Preliminary "range finding" tests gave crude estimates of the LC values at a 4-hour exposure. These results were used as a guide to establish dosages for more extensive experiments in subsequent series. Three different dosages at an exposure period of 4 hours were used in the first extensive series of tests. The second series of tests employed exposure periods of 4, 8, 24, and 48 hours and was designed to provide data for establishing dosage-mortality curves. All dosages were expressed as milligrams per liter (mg./l.) and as micromoles per liter (μM./l.)[3] when calculated on the basis of a 20-liter volume for each fumatorium.

FIG. 1.—Test insects were held in wire screen cages during fumigation in 20-liter bottles as shown.

Amount of Fumatoria Volume Variation.—The average volume per bottle was 19.49 liters. Each bottle was calibrated and the range of volumes was found to be from 19.30 to 20.00 liters, with a standard deviation from the mean of 0.16 liters, or less than 1%. Thermal conductivity gas analyses in the preliminary tests demonstrated that concentrations which existed at the end of each 4-hour exposure period were closer to the values calculated on the basis of 20-liter volumes than when calculated according to the actual volumes. It was assumed that this slight discrepancy was due to sorption and other minor losses.

Test Insects.—Adult confused flour beetles (*Tribolium confusum* Duv.), adult saw-toothed grain beetles (*Oryzaephilus surinamensis* (L.)), and all stages of the rice weevil (*Sitophilus oryza* (L.)) were used. They were reared at 80° F. and 65% relative humidity in sifted-wheat shorts +5% yeast, rolled oats, and Hard Red Winter wheat, respectively. All culture media were tempered to 12.5%

[3] Micromoles per liter = (grams of fumigant/molecular weight ÷ volume of fumatorium in liters) (10^6); one mg./l. = 7.6 μM/l.

moisture before introduction of the parent insects. The cultures were changed every 7 days, therefore, the insects' age could be determined within ±3.5 days. The age of the adults at fumigation was 14 days after emergence from the pupal stage or from the kernels. The three age groups of rice weevil immatures were: RW_i-1, 3.5 days after oviposition (eggs and young larvae); RW_i-2, 10.5 days after oviposition (young and middle-aged larvae); and RW_i-3, 17.5 days after oviposition (older larvae and pupae).

Adult insects were separated from the culture media by sifting. An aspirator was used to collect the desired number of insects for each cage. Ten test cages containing 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 adults, respectively, were used in each fumatorium in the first series of tests. Each insect species required a different dosage range, thus it was necessary to fumigate each species separately. The test insects were placed in the test cages without food for a period of 4 to 8 hours prior to the introduction of the fumigant and remained without food until the first mortality count.

Fifty adult insects per cage were used in the second test series because 50 proved to be the most satisfactory number in the first experiment. Ten grams of wheat infested with the desired age-group of rice weevils were placed in each cage for the immature insect studies.

Postfumigation mortality counts of adults were conducted at 0, 5, 10, 15, and 20 days. Test insects were considered alive if any muscular movement was observed under a three-power illuminated magnifier. If there were a question regarding life in individual insects, efforts were made to stimulate them by use of air currents, prodding, bright light, and mild heat before a decision was made. Adult insects were stored in the rearing room in small plastic boxes (about 2 in. square × ¾ in. deep) containing food material until all postfumigation counts were completed (fig. 2). Following an aeration period of approximately 2 hours, the immature rice weevil samples were also stored in plastic boxes and incubated in the rearing room. Post-fumigation mortality counts of immatures were made when the insects were 4, 5, 6, and 7 weeks old, the ages being figured from the time of oviposition.

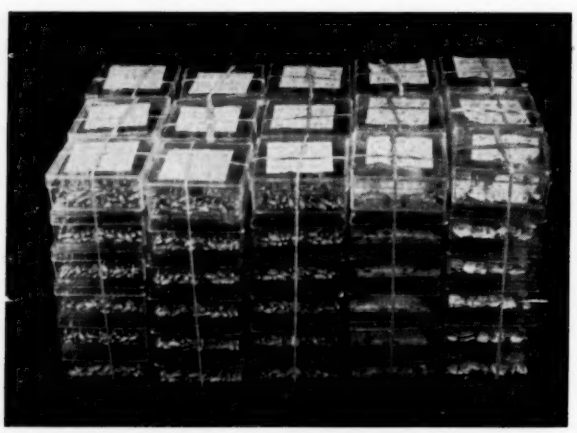

FIG. 2.—After fumigation, the test insects were held at 80° F. and 65% relative humidity in these plastic boxes which contained appropriate food.

Adult insects emerging from the immature test samples were discarded after each count. The differences between numbers emerging from treated samples versus check samples were used as the basis for determining the mortality of the immatures.

The saw-toothed grain beetle is very difficult to work with because of its small size, the ease with which it escapes confinement, its habit of feigning death, its susceptibility to injury from handling, and its very erratic response to treatments. This species was not included in the second series of experiment for these reasons.

Gas Analyses.—Relative gas concentrations were measured at the end of each exposure period by means of a Gow-Mac thermal conductivity gas analyzer. The concentration reading, which was approximately equal to milligrams per liter, was the *sum* of the two components (CCl_4 *and* CS_2) and not a quantitative analysis for each compound. Gas analyses were made only at the end of the fumigation to prevent disturbing or diluting the fumigants during the exposure periods. Each of the gas samples was taken in the vicinity of the test cages, passed through the analyzer, and returned to the top of the fumatorium.

Number of Replicates and Methods of Evaluating Data.—Three insect species in separate cages were used in the preliminary "range finding" tests. These tests consisted of nine dosages in triplicate. Mortality data from these tests served as a guide to dosages which were used in subsequent tests. Results of gas analyses in these tests were used to study the variations related to different bottle volumes and the respective fumigant concentrations resulting from standard dosages.

The first series of tests, to determine the optimum number of adult insects to use per cage, involved triplicate fumigations at three dosages for each of three insect species on three different days. This made a total of nine fumigations (replicates) for each species, dosage, and number per cage. In addition, an untreated check series of three cages for each number of insects per cage was included in each test group. The data were analyzed by the analysis of variance[4] to determine when significant differences occurred between different numbers per cage. The design also made it possible to detect other significant differences such as from one date to another in replicated fumigations, and also from one postfumigation mortality count to the next.

A specific dosage range was used for each group of tests in the second series. These tests were designed to produce data for establishment of dosage-mortality curves and also to support previous information regarding determination of the time when mortality counts should be made. Data from these tests were evaluated by (1) probit analyses to yield LC-values and confidence intervals, (2) graphic analyses to determine when to make mortality counts and (3) tabulation of the minimum dosages in which no insects survived, *i.e.*, 100% mortality.

RESULTS.—*First Series.*—Mortality data most suitable for statistical evaluations are given in tables 1 and 2. Both visual inspection of average mortalities and the analysis of variance suggest that 50 adult insects are sufficient in each test cage (table 1). No distinct demarca-

[4] Gary F. Krause, Instructor in Statistics and Entomology, Kansas State University performed the analysis of variance tests.

Table 1.—Per cent mortality obtained in tests to determine the optimum numbers of adult insects to use per test cage in 4-hour fumigations with 80:20 (CCl_4:CS_2 by vol.) at 80° F.

No. and Date of Test	Insects per Cage																				L.S.D. at the 5% Level
	10		20		30		40		50		60		70		80		90		100		
Rice weevil with 1,161 μM/l.[a]																					
1st test (3/11/57)	95.4	*	88.8	*	94.8	ns	95.8	ns	94.6	ns	96.2	*	93.4	*	96.6	ns	95.2	ns	95.0		
2nd test (3/18/57)	84.4	*	98.0	ns	97.4	ns	95.8	*	91.2	*	96.0	ns	95.4	ns	94.6	ns	92.8	*	89.8		
3rd test (4/1/57)	100.0	ns	100.0	ns	100.0	*	95.8	*	98.8	ns	100.0	ns	98.8	ns	98.8	ns	97.0	ns	97.8		2.2
Range	15.6		11.2		5.2		0.0		7.6		4.0		5.4		4.2		4.2		8.0		
Confused flour beetle with 1,045 μM/l.																					
1st test (3/7/57)	39.0	*	49.6	*	60.2	ns	60.2	ns	64.6	*	80.1	ns	78.0	ns	73.2	*	82.6	ns	86.6		
2nd test (3/25/57)	69.6	ns	71.8	*	79.4	*	87.4	ns	87.8	ns	91.2	ns	91.6	ns	94.0	ns	94.6	ns	93.8		
3rd test (4/15/57)	72.2	*	90.0	ns	91.2	ns	93.2	ns	94.6	ns	98.4	ns	98.2	ns	98.2	ns	98.0	ns	98.4		6.1
Range	33.2		40.4		31.0		33.0		30.0		18.3		20.2		25.0		15.4		11.8		
Saw-toothed grain beetle with 1,567 μM/l.																					
1st test (3/22/57)	84.6	ns	83.6	*	87.6	*	92.2	*	79.2	*	86.0	ns	87.4	ns	86.2	*	82.6	ns	82.4		
2nd test (4/29/57)	90.2	ns	91.2	*	83.4	*	88.4	ns	90.8	ns	93.8	*	88.6	ns	88.2	ns	89.0	ns	89.0		
3rd test (5/11/57)	93.8	*	81.6	*	86.6	ns	84.0	*	87.8	*	82.4	*	86.8	*	83.0	ns	84.4	ns	86.2		3.3
Range	9.2		9.6		4.2		8.2		11.6		11.4		1.8		5.2		6.4		6.6		

[a] Micromoles per liter.
* Significant difference at the 5% level. ns = no significant difference at the 5% level.

tion occurred to support this number in every instance. The frequency of significant differences between number of insects per cage above 50 was lower than the frequency of differences below 50, when considering each test individually. Furthermore, a preferable number of 50 insects is indicated when 30 insects and 100 insects per cage are compared with 50. In this case there were significant differences four times of the nine comparisons between 30 insects and 50 insects per cage, while mortality differences between 100 insects and 50 insects per cage were significant only once in nine comparisons. These comparisons are not specifically indicated in table 1.

Table 2.—Mean per cent mortality obtained in tests to determine when to terminate mortality counts of adult insects following a 4-hour fumigation with 80:20 (CCl_4:CS_2 by vol.) at 80° F.

	Days After Fumigation										L.S.D. at the 5% Level
	0		5		10		15		20		
Rice weevil											
871 μM/l.[a]											
1st test (3/11/57)	73.6	*	53.4	*	69.7	ns	72.5	ns	72.7		
2nd test (3/18/57)	66.0	*	50.0	*	70.3	ns	69.7	ns	70.1		
3rd test (4/1/57)	58.6	ns	58.8	*	82.5	ns	86.9	ns	86.4		5.9
Range	16.0		8.8		12.8		17.2		16.3		
1,161 μM./l.											
1st test (3/11/57)	99.9	*	91.5	ns	93.0	ns	94.1	ns	94.4		
2nd test (3/18/57)	99.1	*	88.8	*	93.3	ns	93.3	ns	93.3		
3rd test (4/1/57)	99.8	ns	98.4	ns	99.0	ns	99.0	ns	99.1		1.6
Range	0.8		9.6		6.0		5.7		5.8		
Confused flour beetle with 1,045 μM./l.											
1st test (3/7/57)	53.1	ns	48.9	*	76.0	ns	80.1	ns	80.5		
2nd test (3/25/57)	70.8	*	83.7	ns	87.1	*	93.2	ns	95.8		
3rd test (4/15/57)	89.1	ns	92.0	ns	94.6	ns	95.0	ns	95.5		4.3
Range	36.0		43.1		18.6		14.9		15.0		
Saw-toothed grain beetle with 1,567 μM./l.											
1st test (3/22/57)	99.7	*	76.4	*	81.0	*	84.0	ns	84.8		
2nd test (4/29/57)	94.5	*	84.0	*	88.8	ns	89.8	ns	89.2		
3rd test (5/11/57)	97.3	*	71.5	*	84.2	*	88.5	ns	86.8		2.3
Range	5.2		12.5		7.8		5.8		4.4		

[a] Micromoles per liter.
* Significant difference at the 5% level. ns = No significant difference at the 5% level.

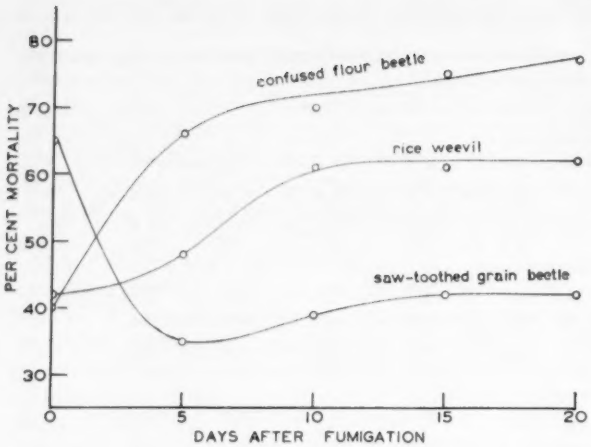

FIG. 3.—Mortality of test insects at different intervals after fumigation with 80:20 (CCl_4:CS_2) at 80 F. (Mortalities are averages from different dosages and exposures.)

The range in mortalities among the three tests (table 1) shows that highly significant differences occurred when identical treatments were used on different dates. The maximum range was 40.4% with the confused flour beetle at 20 per cage and the minimum was zero for the rice weevil at 40 per cage. Mortalities tended to be highest in the third test and lowest in the first test. Hence, a comparison of different numbers per cage within each test served as the basis for determining the minimum satisfactory number of insects per cage.

Table 2 and figure 3 present data which show how long the adult insects should be held after fumigation for final mortality counts. Results in figure 3 for the three species cannot be compared directly because the dosages were different. In table 2, as in table 1, mortalities obtained following fumigation tests on three separate days were usually significantly different. In table 2 the maximum range of mortalities between tests was 43.1% for the confused flour beetle at the 5-day count, but it lessened to 18.6% for the same insects at the 10-day count. The minimum range, 0.8%, was found with the rice weevil in the first count. When comparing mortalities at 0 and 5 days, 0 and 10 days, and 5 and 10 days following the removal of insects from the fumigation chamber, differences were generally significant for adult rice weevils. They were not significantly different after 10 days. Although the 15- and 20-day counts for the flour beetles were higher than the 10-day count, the analysis of variance indicated that these differences were not significant except in one case. Ten days appears to be the most feasible holding period for final mortality counts with the rice weevil and the confused flour beetle. The saw-toothed grain beetle responded differently from the other species in that significant differences were found between 10- and 15-day counts in two of the three tests as shown in table 2. Results of 15- and 20-day counts were not significantly different. Therefore, for final mortality count the saw-toothed grain beetle may be held for 15 days after fumigation with 80:20.

The anesthetic, recovery, and delayed mortality effects are indicated in table 2 and figure 3 as evidenced by the

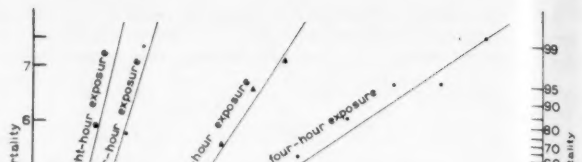

FIG. 4.—Toxicity of 80:20 (CCl_4:CS_2) to adult confused flour beetles.

apparent high "mortality" at zero days, low mortality at 5 days, and increased mortalities at 10, 15, and 20 days. This phenomenon was especially noticeable with the adult saw-toothed grain beetle and was less noticeable with the adult rice weevil in some tests (table 2) but not following completion of all tests (fig. 3). Adult flour beetles did not show an anesthetic response in these tests.

Second Series.—LC_{50} and LC_{90} values with their respective confidence intervals are given in table 3. These values are based on data presented in the probit-mortality lines in figures 4 to 9 inclusive. Figure 9 portrays the relative susceptibility of each species and stage exposed for 24 hours. This relative susceptibility was not constant over the entire mortality range. In the 24-hour exposures (fig. 9) and above 95% mortality, the increasing order of resistance was: rice weevil adults, confused flour beetle adults, middle-aged rice weevil larvae (RW_i-2), rice weevil eggs and young larvae (RW_i-1), and rice weevil pupae and older larvae (RW_i-3). The order of resistance also varied with the different lengths of exposure (figures 4 to 8 inclusive). The immature rice weevils were especially difficult to kill with short exposures (figures 6 to 8 inclusive).

Some of the confidence intervals in table 3 indicate mortalities above 100%. This means that dosages which produced 90% kills, according to the probit-mortality lines were, in some instances, more than needed to produce 100% mortality. This reflects the wide limits of variation in the results of some of these tests. The confidence intervals show that variation was greater in the

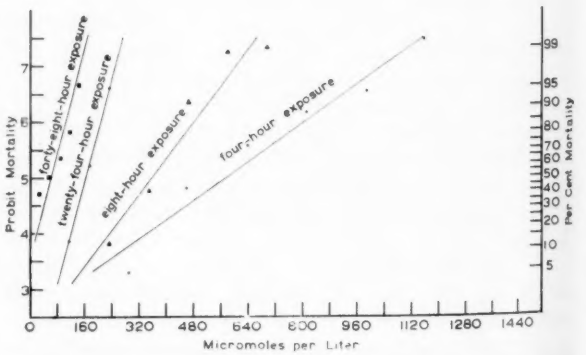

FIG. 5.—Toxicity of 80:20 (CCl_4:CS_2) to adult rice weevils.

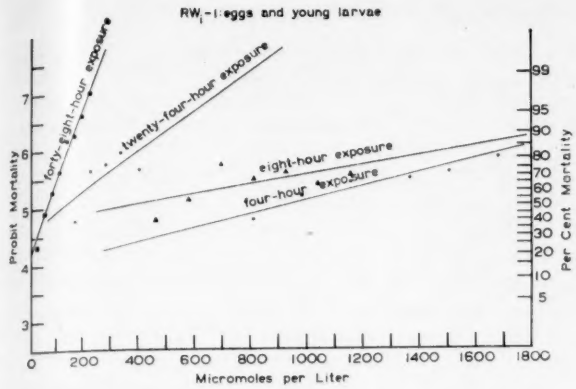

FIG. 6.—Toxicity of 80:20 (CCl_4:CS_2) to immature rice weevils (RW$_i$-1: eggs and young larvae).

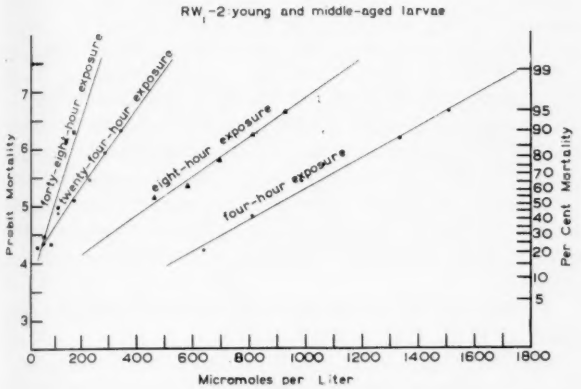

FIG. 7.—Toxicity of 80:20 (CCl_4:CS_2) to immature rice weevils (RW$_i$-2: young and middle-aged larvae).

longer exposures with adult insects. No clearly defined pattern of variation was evident with immature rice weevils. Rice weevil adults demonstrated the most erratic results and middle-aged rice weevil larvae (RW$_i$-2) the least. The youngest rice weevils (RW$_i$-1) were unpredictable in their response to exposures of 4, 8, and 24 hours,

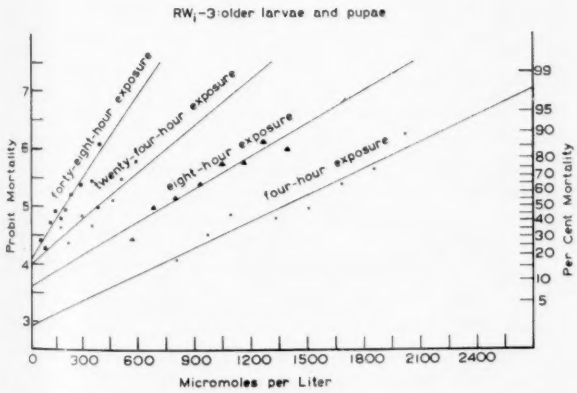

FIG. 8.—Toxicity of 80:20 (CCl_4:CS_2) to immature rice weevils (RW$_i$-3: older larvae and pupae).

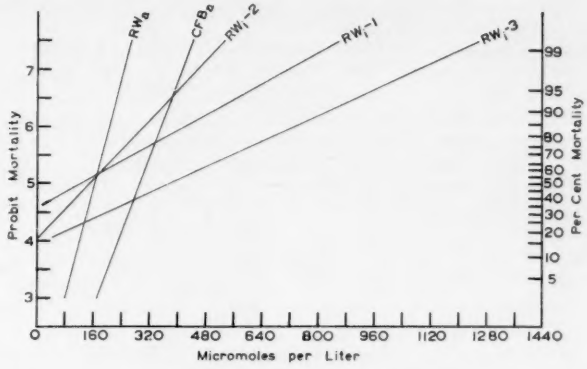

FIG. 9.—Relative toxicity of 80:20 (CCl_4:CS_2) to adult confused flour beetles and four age groups of rice weevils when exposed 24 hours at 80° F.

but their confidence intervals were relatively narrow in the 48-hour exposures.

Table 4 presents the combinations of minimum dosages for each exposure period with which 100% mortalities were associated. The highest dosage tested did not produce a 100% mortality in some tests. In such cases the maximum dosage used was recorded with the observed percentage kill in parentheses. Frequently a lower dosage produced a higher mortality than a higher dosage, even

Table 3—Relative amount of 80:20 (CCl_4:CS_2 by vol.) fumigant needed to produce LC_{50} and LC_{90} mortality in three species of test insects when fumigated for different lengths of exposure at 80° F.

Exposure Period (Hours)	LC_{50} Dosage Required Mg./l.[a]	LC_{50} Dosage Required μM./l.[b]	LC_{50} 95% Confidence Interval (% Mortality)	LC_{90} Dosage Required Mg./l.	LC_{90} Dosage Required μM./l.	LC_{90} 95% Confidence Interval (% Mortality)
Confused flour beetle adults						
4	123	935	±15.3	174	1,320	±16.1[c]
8	89	680	±15.8	107	815	±19.4
24	38	290	±16.7	49	370	±26.9
48	27	205	±20.2	35	265	±40.9
Rice weevil adults						
4	76	575	±19.2	115	872	±23.0
8	47	360	±29.1	68	520	±36.3
24	22	165	±30.0	29	220	±42.9
48	8	62	±38.2	16	120	±50.4
Rice weevil immatures						
RW$_i$—1[d]						
4	121	920	±10.9	263	2,000	±15.2
8	45	340	±21.8	250	1,900	±37.6
24	16	125	±39.9	66	500	±41.0
48	9	65	± 9.8	22	170	± 9.0
RW$_i$—2[e]						
4	118	900	± 8.1	181	1,375	± 9.8
8	79	600	± 9.0	109	830	± 9.1
24	20	155	± 3.3	45	345	± 4.9
48	13	98	± 8.7	25	188	±17.3
RW$_i$—3[f]						
4	180	1,370	± 4.6	292	2,220	± 9.3
8	98	745	± 8.0	188	1,425	±10.3
24	49	375	± 6.3	114	865	±18.4
48	26	195	± 3.7	61	460	± 9.2

[a] Milligrams per liter.
[b] Micromoles per liter.
[c] Numbers in which LC_{90} plus confidence interval total more than 100% mortality indicate that dosages which produced 90% kills according to the probit-mortality curves were, in some instances, more than enough to produce 100% mortality.
[d] Eggs and young larvae.
[e] Young and middle-aged larvae.
[f] Older larvae and pupae.

Table 4.—Minimum dosages of 80:20 (CCl_4:CS_2 by volume) associated with 100% mortality of test insects fumigated in empty 20-liter bottles at 80° F.

Species and Stage	Length of Exposure and Dosage Required							
	4 Hours		8 Hours		24 Hours		48 Hours	
	Mg./l.[a]	μM/l.[b]	Mg./l.	μM/l.	Mg./l.	μM./l.	Mg./l.	μM/l.
Confused flour beetle adults	244	1,858	138	1,045	61	464	34	261
Rice weevil adults	222	1,684	107	813	38	290	23	174
Rice weevil immatures								
RW_i—1[c]	290 (80%)[f]	2,206	244 (66%)	1,858	107 (90%)	813	46	348
RW_i—2[d]	290	2,206	199	1,509	107 (98%)	813	31	232
RW_i—3[e]	290 (90%)	2,206	244 (98%)	1,858	107 (96%)	813	69 (98%)	522

[a] Milligrams per liter.
[b] Micromoles per liter.
[c] Eggs and young larvae.
[d] Young and middle-aged larvae.
[e] Older larvae and pupae.
[f] Mortality did not reach 100%. Mortality at indicated dosage is given in parentheses.

within the same test. In these instances the dosage recorded in table 4 is not the lowest which produced 100% mortality, but rather was the first in the series of dosages in which no less than 100% mortality occurred in subsequent higher dosages. Table 4 and figures 4 to 9 show that exposures of 48 hours or longer should be used to obtain approximately 100% kill of immature rice weevils.

The retarding effect of sublethal fumigation on the development of immature rice weevils is shown in figure 10. At the age of 5 weeks the average emergence was 23% completed in the fumigated samples and 40% completed in the checks. These percentages were based on the total number of adults which appeared in the fumigated and check samples, respectively, at 7 weeks of age.

Discussion.—Although results of analysis of variance were not sharply defined, it appears that an increase in the number of test insects beyond 50 per cage would result in diminishing returns when considering the practical difficulties involved in handling large numbers of insects. The use of 50 insects per cage is convenient in calculating results.

Although the most favorable postfumigation holding periods were clearly indicated for 80:20, experience with other fumigants has demonstrated that each fumigant demands its own special time interval. The anesthetic, recovery, and delayed mortality phenomena are highly important from both the experimental and the practical standpoint. An examination of test insects immediately following a fumigation may result in the conclusion that 100% mortality was attained when actually many were merely anesthetized. If the insects were examined only once, 5 days after fumigation for example, they might appear to be normally active but succumb to the effect of the fumigant later. Therefore, it is desirable to conduct more than one postfumigation examination of the insects to obtain an accurate mortality count.

The range of variation as indicated by the confidence intervals in table 3 was from ±3.3% to 50.4% mortality in the different treatments. There is no apparent way to predict where, within this range, the results of a new set of experiments might fall. This emphasizes the importance of planning experiments with adequate numbers of tests, especially with a design including replicated series on different days. The data should be suitably and conveniently arranged for statistical analyses. It is possible that the use of a reference fumigant having a known performance would aid in evaluation of varying results and give greater reliability.

A relatively long fumigant exposure is particularly important in the treatment of immature rice weevils. It is assumed that this condition is related to the penetration of the fumigant and other factors. This assumption is based in part on the theory that these immature forms, being surrounded by the wheat kernel, are protected from immediate contact with the fumigant vapors. Certain

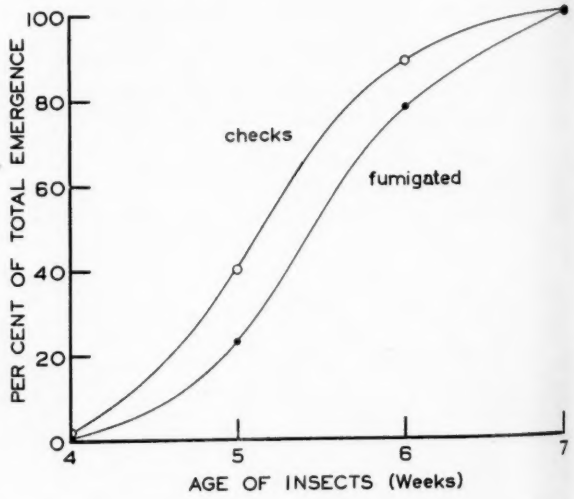

Fig. 10.—Time required for development of immature rice weevils after sublethal fumigation with 80:20 (CCl_4:CS_2) at 80° F. (Emergence counts are averages from different dosages and exposures).

life stages of the insects are less active (metabolically) than others. Several workers, including Cotton (1932), Lindgren (1935), and Sun (1947), have reported various degrees of correlation between rates of metabolism and susceptibility to fumigants. Adkisson (1957) and Krohne & Lindgren (1958) have pointed out the difficulty involved in sorting out the different immature life stages of the rice weevil, especially the older immature stages. Since more than one stage was present in each age group, it is not known precisely which stage was responsible for different parts of the mortality line. Gross estimates of the relative susceptibilities were justified, however, on the premise that each of the three age groups for the immatures represented a fairly narrow band in the total developmental range.

According to mathematical probability a 100% kill is not possible; however it is of great practical importance to estimate the LC_{100} values as in table 4, because those who fumigate stored grain wish to kill as many of the insects as they can with the most efficient use of time and money. This conclusion becomes even more significant in quarantine practices.

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Effect of Temperature on Spotted Alfalfa Aphid Reaction to Resistance in Alfalfa[1]

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ABSTRACT

The effect of different temperatures on the response of spotted alfalfa aphids (*Therioaphis maculata* (Buckton)) was not the same on plants varying in aphid resistance. Aphid survival and reproduction were retarded relatively more at high than at low temperatures on a resistant as compared with a susceptible plant. It appeared that a resistant plant on which an aphid population could not be maintained at 80° would support a limited population at 60° F.

Temperature influenced the reaction of adults and nymphs on resistant, intermediate, and susceptible plants in numerous ways. Optimum temperature for reproductive period, total reproduction of adults, and numbers of nymphs which matured and reproduced decreased with an increase in resistance of the plants. Rates of reproduction decreased with a decrease in temperature and with an increase in resistance of plants, and differences among plants were evident at all except the lowest temperature. A postreproduction period occurred on the susceptible plant at temperatures from 60° to 90° F. but was not evident on the resistant and intermediate plants. The time required for nymphs to mature and reproduce was longer at 60° than at 80° F. and appeared to be longer with an increase in resistance of the plants. Where resistance limited survival, adults appeared to survive longer than nymphs.

Infested seedlings from both resistant and susceptible lines were killed more rapidly at high than at low temperatures, although final survival did not appear affected by temperature, indicating that resistant seedlings would perform satisfactorily under aphid infestation at cool temperatures.

Temperature is known to affect the expression of various genetic factors in certain organisms. Included in these effects is the expression of resistance of plants to insects. To cite two examples, Dahms & Painter (1940) demonstrated that temperature affected the reaction of certain alfalfa plants to pea aphids (*Macrosiphum pisi* (Harr.)), and Cartwright *et al.* (1946) reported a similar phenomenon for hessian fly (*Phytophage destructor* (Say)) resistance in wheat varieties with certain genes for resistance.

Although alfalfa plants resistant to spotted alfalfa aphids (*Therioaphis maculata* (Buckton)) are readily attainable, Hackerott *et al.* (1958), there have been indications that resistance may be less effective at low than at high temperatures. For example, Harvey & Hackerott (1956) reported a low rate of aphid survival at an average temperature of 75° F. on resistant compared with susceptible plants selected from Buffalo alfalfa. However, when subsequent tests at lower temperatures showed increased survival of aphids on resistant plants, the present study was initiated. Similarly, Howe & Smith (1957) noticed that spotted alfalfa aphid nymphs unable to survive on

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resistant plants were able to complete development on these plants in the field at cool fall temperatures.

Although little is known regarding the effect of temperature on the response of spotted alfalfa aphids on resistant plants, the effects of seasonal temperatures on their reaction to susceptible plants have been reported by Dickinson *et al.* (1955) and Harpaz (1955). They found that development and reproduction took place at a reduced rate at lower temperatures.

This investigation was designed to compare the influence of differential temperatures on spotted alfalfa aphid reproduction and development on susceptible, intermediate, and resistant alfalfa clones and to determine the effect of temperature on survival of aphid-infested seedling progenies of resistant and susceptible plants. It was anticipated that data obtained would indicate whether resistance could be expected to be effective during the cool temperatures prevailing in early spring and late fall.

MATERIALS AND EQUIPMENT. Three alfalfa clones—78, 247, and BS 10—representing resistant, intermediate, and susceptible classes, respectively, were used to measure reproduction of adults and development of nymphs. These plants were selected from the susceptible variety Buffalo and classified for resistance by measuring reproduction at 75° F. by a caged-leaf method. Clone 78 was the most resistant selection available; and although BS 10 was not selected for extreme susceptibility, it appeared about average in susceptibility for the variety Buffa'o. Clone 247 was chosen from a group of clones less resistant than 78 but not so susceptible as BS 10 to represent the intermediate class. Although aphid reaction to clone 247 at 75° F. more nearly approximated the resistant than the susceptible class, it seemed probable that clones less resistant than 247 would not serve so well to represent the intermediate class at low temperatures.

The leaf-cage assembly showing an infested trifoliate is illustrated in figure 1. A 3-inch section of stem with a single trifoliate taken from new growth of the three test clones growing in the greenhouse was inserted into a 23-×85-mm. shell vial which was filled with water and covered with masking tape. The glass tube enclosing the trifoliate was held in position with strips of masking tape, which were readily removed for extracting nymphs in the reproduction study. The open end of the leaf cage was covered with nylon cloth fastened with a rubber band.

Excised rather than attached leaves were used to facilitate daily manipulation and counting of aphids. In preliminary trials resistant, intermediate, and susceptible clones remained in the same resistance class whether tested as excised or attached leaves. Comparable results in spotted alfalfa aphid reaction between cuttings and plants growing in the field were obtained by Howe & Smith (1957). Cuttings have also been used by Padilla & Young (1958) in studying the biology of the spotted alfalfa aphid.

In the seedling survival trial, the effect of two temperature levels on the mortality of the susceptible variety Buffalo was compared with the bulked polycross progenies

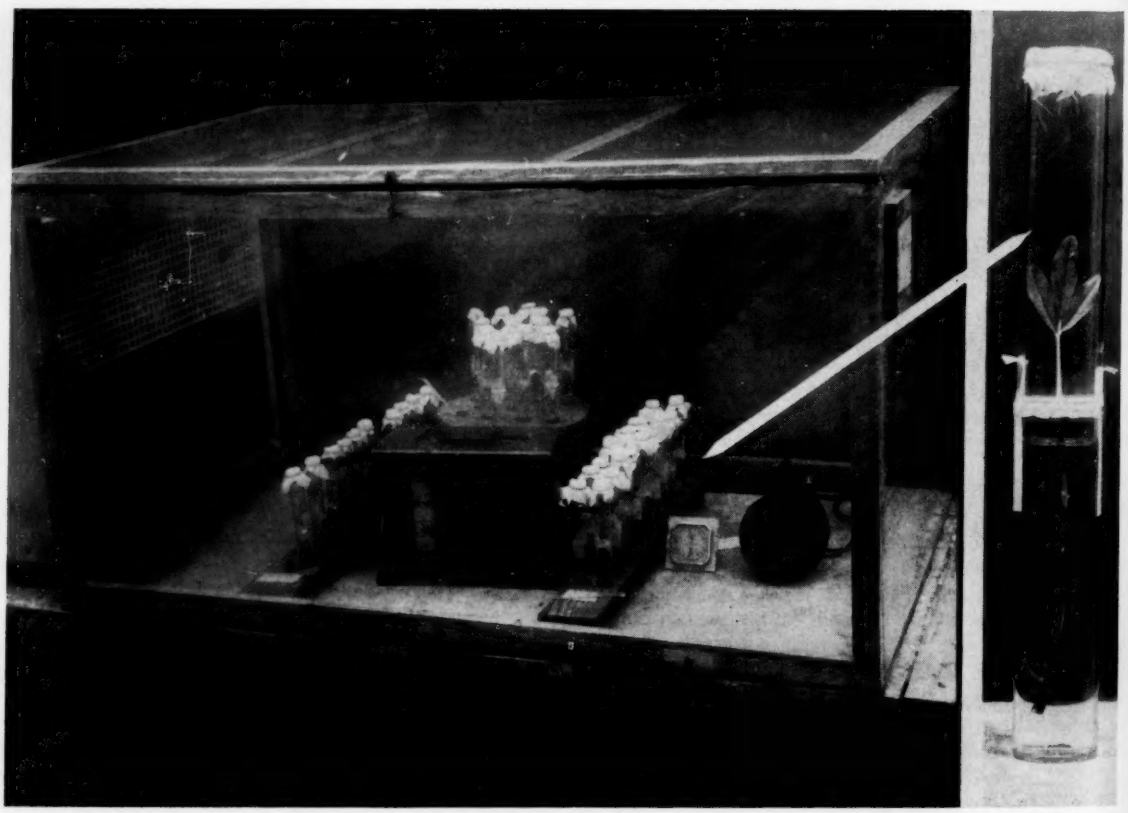

FIG. 1.—Temperature control chamber with "window-type" air conditioner. Cage used to maintain aphids on excised leaves shown at right.

of 12 resistant clones selected from Buffalo.

Temperatures were controlled by use of double-walled plastic chambers 2×2½×4 feet. Chambers maintained at 60° and 70° F. were equipped with "window-type" air conditioners as illustrated in figure 1. The greenhouse temperature was maintained at 75° F. and two additional chambers were maintained at 80° and 90° F. with thermostatically controlled electric heaters. Continuously operated blowers kept temperatures within each cage uniform. All chambers were exposed to equal amounts of natural light at day lengths prevailing during March, April, and May. Although the equipment was not designed for precise temperature control, the ±5° F. variation in temperature was regarded as sufficiently accurate for the purpose of these studies. Relative humidity was not controlled but was undoubtedly high within the leaf cages.

The aphids used in these tests were obtained from greenhouse cultures reared at about 75° F. on susceptible BS-10 clones. By appropriate transfers, adults in these tests were used within 18 hours after reproducing for the first time and nymphs studied were used within 18 hours after birth. Aphids were induced to move onto the test leaves with the aid of a camel's hair brush.

METHODS AND PROCEDURE.—Survival and reproduction of adults were determined on three classes of alfalfa plants, namely; resistant-, intermediate-, and susceptible-to-spotted-alfalfa aphids. These classes of plants were compared at four temperatures (60°, 70°, 80°, and 90° F.). Each clone was replicated eight times within each temperature level. A young reproducing adult was introduced on a caged leaf of each clone at all temperatures and the nymphs produced by each adult were recorded and removed daily until death of the adult. Daily removal of the nymphs increased accuracy of counts and aided in keeping the leaves in good condition. Data were obtained on length of reproductive period, total reproduction, rate of reproduction, and length of post reproduction period.

The development and survival of nymphs on the same three classes of plants were compared at two temperatures, 60° and 80° F. Newly born nymphs were caged on 50 leaves of each class of plant at each temperature. The presence of nymphs was recorded daily until they died or matured and reproduced. This procedure permitted obtaining data on the numbers of nymphs maturing and reproducing and the time required to mature and reproduce, as well as the survival of nymphs prior to reproduction.

To record aphid development, leaf cages were removed from the control chamber for not more than 5 minutes each day. Leaves showing signs of deterioration before completion of a trial were replaced with new leaves from the same clone in a few instances.

Percentages of survival of seedlings of susceptible variety Buffalo were compared with survival percentages of polycross progenies of resistant selections from Buffalo at 60° and 80° F. Susceptible and resistant entries were seeded in alternate rows in flats in 12 replications. Two weeks after planting approximately 1,500 seedlings per entry were placed in each temperature chamber and heavily infested by scattering spotted alfalfa aphids over the flats. Surviving seedlings were counted 2, 4, and 6 weeks after infestation.

RESULTS.—*Adult Reproduction.*—The mean reproduction period in days and total as well as rate of reproduction of eight apterous adults on each of three classes of plants at four temperatures are shown in table 1. Length of reproductive life was significantly shorter on the resistant clone than on the susceptible, regardless of temperature, and significantly less than the intermediate clone at the two lower temperature levels, 60° and 70° F. Length of reproductive life was significantly longer on susceptible than the intermediate clone at all temperature levels. As the temperature decreased, length of reproductive period increased on all classes of plants. However, there was a significant interaction of clones×temperatures. Thus the reproductive period on resistant and intermediate clones was relatively longer than for the susceptible clone at low compared with high temperatures. For example, in comparing the response of 60° vs. 80° F., the length of reproductive life on the susceptible clone was about twice as long at 60° as at 80° F. while the corresponding increase on the resistant clone was more than 10-fold.

Although reproductive period was significantly longest at 60° F. on all classes of plants, total reproduction per adult was significantly highest at 70° and 80° F. for the

Table 1.—Effect of temperature on reproduction of 8 apterous adult aphids on 3 classes of alfalfa clones (78 resistant; 247 intermediate; BS 10 susceptible).

Temperature	Reproductive Period (Days)					Total Reproduction (Nymphs/♀)					Rate of Reproduction (Nymphs/♀/Day)				
	Resistant		Intermediate		Susceptible	Resistant		Intermediate		Susceptible	Resistant		Intermediate		Susceptible
60	22	** [a]	30	**	49	31	**	46	**	71	1.4		1.5 [b]		1.6
	**		**		**	**				**					
70	5	**	20	**	35	8	**	43	**	95	2.1		2.2		2.9
			**		**			**							
80	2		4	**	23	3		10	**	93	2.2		2.5	*	4.1
					*					**					
90	1		1	**	17	2		3	**	58	1.6	*	3.4		3.5

[a] Figures separated by asterisks are significantly different (**=1% level, *=5% level).
[b] A break in the vertical line denotes 5% level of statistical significance between temperatures.

Table 2.—Effect of temperature on development of 50 spotted alfalfa aphid nymphs on each of 3 classes of alfalfa plants (78 resistant; 247 intermediate; BS 10 susceptible).

Class of Plants	Nymphs Alive on 5th Day		Nymphs Maturing and Reproducing		Average Days Survived Prior to Reproducing		Days Required to Mature and Reproduce	
	60° F.	80°F.	60° F.	80° F.	60° F.	80° F.	60° F.	80° F.
Resistant	25	0	5	0	8	1	21	—
Intermediate	43	14	25	9	14	2	20	7
Susceptible	49	49	45	48	17	6	19	6

susceptible clone, 60° and 70° F. for the intermediate, and 60° F. for the resistant clone (table 1). Significantly more nymphs per female were produced on the susceptible clone than on the resistant and intermediate clones at all temperatures. Likewise, more nymphs per adult were produced on the intermediate than on the resistant clone at all temperatures, but differences in reproduction were not significant except at the lower temperature levels, 60° and 70° F. The relative ranking of the three clones on total nymph production did not change regardless of temperature, but the interaction of clones×temperatures was significant at the 1% level. The significance of the interaction appears to be due to the marked increase in reproduction on resistant clone at 60° F. and on the intermediate clone at 70° and 60° F. While reproduction on the susceptible clone was highest at 70° and 80°, it exhibited a marked decline at 60° F. and 90° F. The increased total reproduction on resistant and intermediate clones at lower temperatures may be attributed to an increase in number of days of adult reproductive period at 60° and 70° F. On the other hand, the greatest reproduction on the susceptible clone occurred at 70° and 80° F. because the increase in rate of reproduction at these temperatures was accompanied by only a moderate decrease in length of reproductive period. Also, total reproduction was lowest at 90° F. since the decrease in length of reproductive life was sufficient to offset the high rate of reproduction.

The interaction of clones×temperatures with respect to rate of reproduction (nymphs per adult per day) was highly significant. The rate of reproduction increased on the resistant clone with an increase in temperature up to 80° F., but not significantly. Reproduction rate was significantly higher at 90° than at 60° F. on the intermediate clone, and the rate of reproduction at 80° and 90° was significantly higher than at 60° F. for the susceptible clone. Significant differences in rates of reproduction could not be demonstrated among the clones at 60° F., but rates tended to be lower with an increase in resistance of the clones. Although rates of reproduction at all temperature levels were greater on susceptible than on the intermediate clone, the only significant and marked increase in rate was at 80° F. The rate of aphid reproduction was higher on intermediate than on the resistant clone at all temperatures, but differences were significant only at 90° F. However, the rate of reproduction was difficult to determine on resistant and intermediate clones at high temperatures because of the short period of reproductive life.

A postreproduction period, not included in the table of data, on the susceptible clone averaged 7 days over all temperatures. Harpaz (1955) reported a similar postreproduction period for spotted alfalfa aphids on susceptible plants. In contrast, adults did not survive longer than 1 day on resistant and intermediate clones after reproduction ceased.

Nymph Survival and Development.—The reaction of nymphs on resistant, intermediate, and susceptible clones at 60° and 80° F. is shown in table 2. A chi-square test for independence showed a significant interaction of clones× temperatures for the survival of nymphs (numbers alive out of 50 nymphs on fifth day) and for the numbers of nymphs which matured and reproduced. Although nymphal survival was lower on resistant and intermediate clones than on a susceptible clone at both temperatures, the relative reduction in survival was greater on the resistant and intermediate clones at 80° than at 60° F. The numbers of nymphs becoming adults appeared about equal on the susceptible clone at the two temperatures. In contrast, the intermediate clone had three times as many nymphs reaching adult stage at 60° as at 80° F.; and although no nymphs matured on the resistant clone at 80° F., 5 of the 50 nymphs matured and reproduced at 60° F. The time required to reproduce was about three times as long at 60° as at 80° F., and appeared to increase with an increase in resistance of the clones.

It appeared that adults were capable of surviving longer than nymphs on the resistant clone. For example, the adults reproduced for 22 days on the resistant clone at 60° F. (table 1) while nymphs survived 8 days on the same clone at the same temperature (table 2).

Seedling Survival.—Percentages of survival of seedling progeny of resistant and susceptible plants at 60° and 80° F. are compared at 2, 4, and 6 weeks after aphid infestation in table 3. Mortality of seedlings was more rapid at 80° than at 60° F. in both classes of plants. An analysis of variance of percentage data, transformed to arcsin scale, on the 14th day after infestation showed a significant interaction of classes of seedlings×temperature. This was

Table 3.—Effect of temperature on percentages of survival of resistant and susceptible alfalfa seedlings following infestation with spotted alfalfa aphids.

Class of Seedlings	Per Cent Survival (Days After Infestation)					
	14		28		42	
	60° F.	80° F.	60 F.	80° F.	60° F.	80° F.
Resistant[a]	96	89	66	46	49	44
Susceptible[b]	56	14	2	1	0.2	0.8

[a] 1,500 polycrossed progenies of 12 clones resistant to spotted alfalfa aphids.
[b] 1,500 seedlings of the variety Buffalo.

due to relatively greater mortality of seedlings at 80° F. compared with 60° F. for the susceptible class than for the resistant seedling progenies. Seedling progeny of resistant plants were significantly higher in percentage survival at 60° than at 80° F. on both the 14th and 28th days and on the susceptible seedlings on the 14th day. Final survival counts on the 42nd day approached zero for susceptible Buffalo seedlings at both temperatures and varied from 44% at 80° F. to 49% at 60° F. for the resistant class. The latter differences were not significant.

DISCUSSION.—The advantage of resistant and intermediate over a susceptible plant in retarding spotted alfalfa aphid reproduction and development was relatively greater at high than at low temperatures. Although it may be desirable to evaluate the effect of lower temperatures, aphid reproduction and development were sufficiently retarded at 60° F. on the resistant clone that resistance may remain effective. That normal development of adults was inhibited by resistant and intermediate plants at all temperatures was also shown by lack of a postreproduction period on these plants. Nearly equal survival of seedling progeny of resistant plants at 60° and 80° F. indicated probable satisfactory performance of resistance at low temperatures under aphid infestation.

The three clones chosen to represent susceptible, intermediate, and resistant classes may not be typical of plants carrying other genetic factors concerned with resistance. However, general observations of many clones of a wide spectrum of resistance indicated that the types of differences reported are those commonly observed.

As in the case of the results of the present study on spotted alfalfa aphids, optimum temperature for pea aphid reproduction and survival according to Dahms & Painter (1940) was also several degrees higher for susceptible than for apparently resistant alfalfa plants. They suggested that a deficiency in available food for pea aphids in resistant plants would be more detrimental at high temperatures, since the metabolic rate of the aphids would be increased. This suggestion may also explain the differential response of spotted alfalfa aphids to temperature on susceptible and resistant plants. Moreover, if resistance were attributable to a toxin, an increased metabolic rate would also appear to be more harmful to aphids at higher temperatures.

Although temperature seems to affect pea and spotted alfalfa aphid resistance similarly, an opposite reaction to temperature for hessian fly resistance occurs. Cartwright *et al.* (1946) showed that fly infestations were relatively greater at high than at low temperatures for certain resistant compared with susceptible wheats. They were unable to determine if the effects of temperature were due solely to response of host plant or a combined response of both host and insect, but they indicated resistance of the host plant was affected. Painter (1951) considered that the effect of temperature on hessian fly resistance may be attributed to the effect of temperature on rate of plant growth.

Because no method was available to maintain aphids and plants at different temperatures simultaneously, it was impossible to determine whether effects of temperature were due primarily to response of the aphids or the plants. However, observations made in conjunction with this study indicated that the expression of resistance in plants preconditioned for 3 weeks at 60° and 80° F. was not affected when evaluated for aphid reaction at a common temperature. Moreover, the resistance expression of a single trifoliate was not altered when the remainder of the plant was maintained at either higher or lower temperatures. Possibly a change in resistance in one part of a plant could not be transferred to another part since it has been demonstrated (Harvey & Hackerott 1958) that spotted alfalfa aphid resistance was not transferred to a susceptible scion on a resistant plant.

In considering the relative merits of evaluating plants for resistance at different temperature levels, it appears that resistant and intermediate plants may be distinguished from susceptible at all temperature levels studied but more readily at high than at low temperatures. To identify degrees of resistance or to separate resistant and intermediate classes, testing at lower temperatures may be more effective for all aphid reactions measured except rate of reproduction. Survival of nymphs appeared to be a more sensitive indicator of resistance than survival or reproduction of adults. It appears that the higher the level of resistance in a plant at high temperatures, the more resistance it will exhibit at low temperatures. However, it may be desirable to test for resistance at both high and low temperatures, especially if plants exist with genes for resistance which are more effective at low than at high temperatures.

Although other environmental factors, such as humidity and light, may also affect the expression of resistance, it is evident that temperature is an important factor in affecting reaction of spotted alfalfa aphids to resistance in alfalfa.

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Screening Tests in 1957 with Synthetic Compounds for Toxicity to House Flies when Applied in Space Sprays[1]

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Abstract

The results of preliminary evaluations of 107 synthetic compounds when applied as space sprays to the house fly, *Musca domestica* L., are reported. Eleven compounds, all but one esters of *dl-cis-trans*-chrysanthemumic acid, phosphoric acid, or a substituted phosphonic acid, were sufficiently toxic to warrant further study.

In connection with the insecticide screening program of the Entomology Research Division preliminary evaluations are made against the house fly, *Musca domestica* L. (Piquett & Gersdorff 1958). The results of such tests with 107 compounds[2] are reported in this paper.

The compounds were tested as solutions in refined kerosene (Deobase) at a concentration of 20 mg. per milliliter. Acetone was sometimes added as an auxiliary solvent, but because these compounds proved to have no or negligible toxicity, no correction was needed for the presence of this solvent.

Sprays containing 1, 2, and 4 mg. of pyrethrins per milliliter of Deobase were used as standards of comparison. Mortalities were determined by the Campbell turntable method in duplicate tests against laboratory-reared house flies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain. About 100 flies averaging 2 to 3 days in age were used in each test.

Eleven compounds caused considerable mortality at the concentration tested. All but one are esters of *dl-cis-trans* chrysanthemumic acid, phosphoric acid, or a substituted phosphonic acid. Their approximate rating in comparison with pyrethrins (100%) is given in table 1. These estimations were obtained in a comparison with the results from the standard sprays against the pertinent population of flies. The first nine compounds knocked down 60% or more of the flies. Further study is warranted with most of these compounds because of high toxicity or chemical relationship or both.

Table 1.—Results of tests with the 11 compounds found most toxic to house flies when applied in space sprays.

Compound	Per Cent Mortality				Per Cent Rating
		Pyrethrins			
	Compound	1 Mg.	2 Mg.	4 Mg.	Compared with Pyrethrins
Chrysanthemumic acid, 6-bromopiperonyl ester	100	21	53	81	40[a]
Phosphonic acid, 1-butyryloxy-2,2,2-trichloroethyl-, dimethyl ester	100	18	37	81	40[a]
Phosphoric acid, diethyl 2,4-dimethylbenzyl ester	97	9	37	79	38
Chrysanthemumic acid, 3,4-dimethylbenzyl ester	90	26	58	79	30
3-allyl-4-methoxybenzyl ester	46	4	21	42	22
Phosphoric acid, diethyl 3,4-dimethylbenzyl ester	67	9	37	79	15
diethyl 2-methylallyl ester	23	9	37	79	7
Chrysanthemumic acid, *o*-(2-methylallyloxy)benzyl ester	30	28	53	84	6
6-nitropiperonyl ester	49	45	68	92	5
3-methoxy-4-(2-methylallyl)benzyl ester	22	28	53	84	4
Acetaldehyde, ethyl phenyl acetal	18	45	58	92	2

[a] Possibly much greater, since test compound caused 100% mortality.

The remaining 96 compounds caused negligible or no mortality. These ineffective compounds represent a number of molecular types and are listed below.

Acetaldehyde,
2-(2-ethoxyethoxy)ethyl 3-(3′,4′-methylenedioxyphenyl)propyl acetal
2-(2-ethoxyethoxy)ethyl α-methylpiperonyl acetal
3-(2-bromo-4,5-methylenedioxyphenyl)-2-methylpropyl isobutyl acetyl
2-(2-butoxyethoxy)ethyl 2-(2-ethoxyethoxy)ethyl acetal
2-(2-butoxyethoxy)ethyl isobutyl acetal
butyl heptyl acetal
butyl 1-methylheptyl acetal
butyl *p*-methoxybenzyl acetal
butyl 2-methyl-3-(3,4-methylenedioxyphenyl)propyl acetal
2-(2-ethoxyethoxy)ethyl heptyl acetal
2-(2-ethoxyethoxy)ethyl 3-methoxybutyl acetal
2-(2-ethoxyethoxy)ethyl *p*-methoxybenzyl acetal
2-(2-ethoxyethoxy)ethyl 1-methylheptyl acetal
isobutyl heptyl acetal
isobutyl *p*-methoxybenzyl acetal
isobutyl *m*-methylbenzyl acetal
isobutyl 1-methylheptyl acetal
isobutyl phenyl acetal
isobutyl 6-propylpiperonyl acetal
phenyl-diethyl acetal
Acetic acid, (3,4-methylenedioxyphenyl)-, methyl ester
Acrylic acid,
3-(2-bromo-4,5-methylenedioxyphenyl)-2-phenyl-, ethyl ester
3-(2-chloro-4,5-methylenedioxyphenyl)-2-phenyl-, ethyl ester
3-(*o*-methoxyphenyl)-2-phenyl-, methyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, ethyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, methyl ester
Acrylamide, *N*,*N*-diethyl-3-*p*-methoxyphenyl-
N,*N*-diethyl-3-(3,4-methylenedioxyphenyl)-2-phenyl-
o-Anisaldehyde, diethyl acetal
p-Anisaldehyde, diethyl acetal
Benzaldehyde,
o-(allyloxy)-, diethyl acetal
p-isopropyl-, diethyl acetal
o-(2-methylallyloxy)-, diethyl acetal
Benzene,
4-[3-(1-isobutoxyethoxy)-2-methylpropyl]-1,2-methylenedioxy-
1,2-methylenedioxy-3,4,5-trichloro-
Bicyclo[2.2.1]hept-2-ene, 5-butoxy-1,2,3,4,7,7-hexachloro-
2-Butanol, 3,4-dibromo-2-methyl-
Chrysanthemumic acid,
3-allyl-4-ethoxybenzyl ester
α-allyl-*p*-isopropylbenzyl ester
2-allyl-4,5-methylenedioxyphenyl ester
2-allyloxy-3-methoxybenzyl ester
2-(3-allyloxy)naphthalenemethyl ester
benzhydryl ester
2-bromo-4,5-methylenedioxyphenyl ester
p-*tert*-butylbenzyl ester

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[2] Synthesized by W. F. Barthel, B. H. Alexander, and R. T. Brown, chemists of the Entomology Research Division.

α-butylpiperonyl ester
α-*tert*-butylpiperonyl ester
α-cyclohexylpiperonyl ester
2,3-dibromo-1,1-dimethylpropyl ester
2-(3-ethoxy)naphthalenemethyl ester
4-methoxy-3-(2-methylallyl)benzyl ester
2-(3-methoxy)naphthalenemethyl ester
3-methoxy-2-propoxybenzyl ester
α-(2-methylallyl)piperonyl ester
6-methyl-3-cyclohexene-1-methyl ester
3,4-methylenedioxybenzhydryl ester
p-methyl-α-propylbenzyl ester
2-naphthyl ester
α-naphthylmethyl ester
α-phenethylpiperonyl ester
phenyl ester
p-phenylbenzyl ester
β-propylpiperonyl ester
2,4,6-trimethyl-3-cyclohexene-1-methyl ester
thymyl ester
Cinnamaldehyde, diethyl acetal
1,1-Cyclobutanedicarboxylic acid, diethyl ester
3-Cyclohexene-1-carboxaldehyde,
diethyl acetal
1-methyl-, diethyl acetal
5-Hexenoic acid, 6-(3′,4′-methylenedioxyphenyl)-4-oxo-, ethyl ester
1-Naphthamide,
N,*N*-diethyl-*N*,*N*-dipropyl-
Phenol,
2-bromo-4,5-methylenedioxy-, acetate
m-methoxy-, acetate
3,4-methylenedioxy-, acetate
2-nitro-4,5-methylenedioxy-, acetate
Phosphoric acid,
allyl diethyl ester
diethyl *m*-methylbenzyl ester
diethyl 2-propynyl ester
Piperitone
Piperonal, diethyl acetal
1-Propanol,3-(2′-bromo-4′,5′-methylenedioxyphenyl)-2-methyl-, acetate
Propionaldehyde,2,2,3-trichloro-, diethyl acetal
Propionic acid,
3-(*o*-methoxyphenyl)-2-phenyl-, methyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, ethyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, methyl ester
3-(3,4-methylenedioxyphenyl)-, ethyl ester
3-(3,4-methylenedioxyphenyl)-, methyl ester
3-(3′,4′-methylenedioxyphenyl)2-phenyl-, ethyl ester
3-(3′,4′-methylenedioxyphenyl)2-phenyl-, methyl ester
Pyran,
tetrahydro-2-(2-allyl-4,5-methylenedioxyphenoxy)-tetrahydro-2-piperonyloxy-
Tropic acid,
β-(3,4-methylenedioxyphenyl)-, ethyl ester, acetate
β-(3,4-methylenedioxyphenyl)-, methyl ester, propionate
β-(3′,4′-methylenedioxyphenyl)-, methyl ester, acetate
Veratraldehyde, diethyl acetal

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The Biology and Seasonal Development of the Vetch Bruchid, *Bruchus brachialis* Fahraeus, in Oregon[1]

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ABSTRACT

The first adults of the vetch bruchid (*Bruchus brachialis* Fahraeus) emerged from hibernation in the Willamette Valley of Oregon during the latter half of April of 1954 and 1955. Adults continued to emerge until field populations reached their peak in late May and the early part of June. Mating occurred shortly after emergence and oviposition began when the first pods appeared on hairy vetch (*Vicia villosa* Roth) from June 10 to 18. Oviposition continued through July into the beginning of August, at which time most of the pods on vetch were mature. The field populations of adult bruchids declined steadily from the time of peak abundance until early August, when none were to be found. The eggs began hatching near the end of June and new larvae continued emerging until early August. The larvae penetrated the pods and began feeding within individual seeds. Larval development was evident within the seeds until late September. The first pupae appeared in mid-August, and the first new generation adults at the beginning of September. Newly emerged adults apparently sought hibernation sites soon after emergence, since they were rarely collected in the field. Many of the adults did not emerge from the seeds in the fall and remained alive in storage bins and granaries until the following spring.

The vetch bruchid, *Bruchus brachialis* Fahraeus, is one of the most serious pests of vetch seed in the United States. The larva destroys the seed by completely devouring its contents. The bruchid is believed to have originated in the Mediterranean area where it fed on various wild species of *Vicia*. In the United States it was first collected in the field in New Jersey in June of 1930. The first Oregon record of this pest was 1936 (Rockwood 1945), and by 1942 it had become sufficiently abundant in this area to limit the production of hairy vetch seed. Ade-

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quate control measures were devised by Rockwood & Reeher (1946), but detailed information on the biology and seasonal development of this insect in Oregon was never reported. This paper presents data collected during 1954 and 1955 in Benton and Polk Counties of Oregon.

EMERGENCE.—In 1954 and 1955, the first adults appeared during the last week of April in the mid-Willamette Valley. The males reached peak abundance in the latter part of May, after which their numbers declined rapidly. Females, on the other hand, were not taken until 10 days after the first males appeared and reached a peak in the latter part of June. Table 1 presents the data on the relative abundance of males and females taken from collections in 12 fields during 1954 and 1955 in Polk County. The numbers of bruchids were determined by averaging the number of adults taken per 100 sweeps in each of the 12 fields.

Although a 1:1 sex ratio was determined on the basis of 1,100 adults reared from larvae during 1954, this ratio was observed in the field for but a few days in the latter part of May.

MATING AND OVIPOSITION.—Matings were observed in the field as soon as females began to emerge, and continued until the second week of June. At this time the first pods appeared and the incidence of males declined sharply. Several attempts to force matings of the bruchids in the insectary were unsuccessful. Large numbers were confined to small cartons so that males and females could not possibly avoid contact, but no matings were observed.

Oviposition was noted as soon as the young pods were present on hairy vetch, and it continued throughout the lives of the females or until the pods had matured. In 1954 this period extended from June 15 until August 4. The duration of oviposition approximated the 6-week period reported by Randolph & Gillespie (1958) in Texas.

The most frequent site of oviposition was along the margin of the valve of the pod, about one-eighth of an inch from the edge. Occasionally eggs were deposited seemingly at random over the pod, with some noted at the base of the pod and others on the petiole. The number of eggs per pod was closely related to the stage of development of the pods in relation to bruchid abundance.

Table 1.—Relative abundance of female and male *Bruchus brachialis*.

Sampling Date	Adults per 100 Sweeps		Sex Ratio	
	Females	Males	Females	Males
May 1	—	7	0:1.00	
May 10	8	27	1:3.38	
May 20	100	220	1:2.20	
May 26	173	208	1:1.20	
May 30	180	172	1:0.96	
June 7	191	102	1:0.53	
June 15	194	68	1:0.35	
June 25	200	33	1:0.17	
July 2	187	14	1:0.07	
July 12	164	11	1:0.07	
July 16	138	2	1:0.01	
July 23	92	—	1:0.00	
July 29	27	—	1:0.00	
Aug. 4	9	—	1:0.00	
Aug. 9	—	—	—	

Examination of numbers of pods of the same maturity taken from the same area indicated that the females oviposited in an erratic manner, for some pods were egg-free while others had as many as 53 eggs.

No oviposition was noted in the field prior to the formation of the hairy vetch pods even though dissection of several females revealed fully formed eggs in the oviducts. However, when ovipositing females were confined to glass tubes lacking vegetative material, eggs were deposited on the glass. This would indicate that there is a limit to the period of egg retention, particularly after oviposition has begun.

Females were collected from the field just prior to the formation of vetch pods and introduced into lantern chimneys containing fresh vetch racemes with pods. The confined females lived from 3 to 13 days and produced an average of 24 eggs each. One female laid 62 eggs during confinement and another laid 25 eggs during a single day. All the females were dissected upon their death and in each specimen both the oviducts and ovaries contained numerous eggs. Our inability to maintain the bruchid under insectary conditions and the absence of knowledge of adult longevity render it impossible to estimate the fecundity of the female. It is likely that the egg laying potential is several times the average number observed in these confinement studies.

INCUBATION.—Two experiments were conducted to determine the incubation period of the eggs of the vetch bruchid. The first was conducted from June 24 to July 27, 1954, using 264 eggs in insectary cages and 35 eggs tagged in the field. The incubation period ranged from 13 to 21 days with a mean of 14.6 in the insectary, and 12 to 19 days with a mean of 15.1 in the field. Temperature records maintained in the insectary showed a mean maximum temperature of 74.9° F., mean minimum of 50.8 ° F., and a mean of 62.9° F.

The experiment was repeated from July 26 to August 9, using 107 eggs in the insectary and 33 in the field. The incubation period ranged from 10 to 15 days, with an average of 11.9 in the insectary, and 9 to 16 days with an average of 12.1 in the field. The insectary temperature records during this period were a mean maximum of 81.1° F., a mean minimum of 52.2° F., and a mean of 66.7° F.

The 15- and 12-day incubation periods differ considerably from the 4- to 5-day period reported in Texas (Randolph & Gillespie 1958), and from the 8 days reported by Pinckney (1937) in New Jersey.

An attempt was made to determine the effect of mean temperatures on the period of incubation. Individual records of the incubation period of each of more than 300 eggs were kept, and the mean temperatures for the incubation of each calculated. The data are presented in table 2. Except for the last six eggs the data illustrate that an increase in the mean developmental temperature causes a decrease in the length of incubation. An exposure to higher mean temperatures was not undertaken. Means above 67° are rare in the area under study.

Egg mortality records were kept in the insectary experiments, and of 331 eggs laid, 60, or 18.1% did not hatch. No similar field records were kept, but observations of many pods in the field indicated that egg mortality ran as high as 50%. Cheng (1947), in studying a

Table 2.—Length of time for egg development with the corresponding mean temperatures ° F.

No. of Eggs	Length of Time for Development (Days)	Mean Temperature (° F.)
5	10	66.4
41	11	66.3
35	12	66.4
81	13	62.3
110	14	62.1
46	15	61.3
19	16	61.5
1	17	62.9
25	18	59.8
2	19	63.3
3	20	63.5
1	21	63.4

bean weevil. *Bruchus* sp., attributed a high mortality of eggs to the direct rays of the sun. Since sunlight was the only apparent variable between field and insectary, it may well be the cause of the higher egg mortality in the field.

Larval Development. The fully formed larva within the egg bores directly through the chorion of the egg and the valve of the pod to gain entry. In 1954 the first eggs hatched during the last few days of June. Hatching of the eggs continued until mid-August. The newly hatched larva enters a seed by chewing a tiny hole in the seed coat. The larva remains within the seed throughout the rest of its development.

The mean widths of the head capsules of each of the four instars corresponded very closely with those reported by Randolph & Gillespie (1958) but the range of variation within each instar of the Oregon material was not so extensive.

To determine the developmental rate of the larval instars, 200 field pods with newly laid eggs were tagged. These pods were observed daily until the first egg had hatched on each pod, at which time the remaining eggs were removed. Several pods were removed each day after hatch of the eggs and taken into the laboratory. The seeds were then dissected and the included larval head capsule measurement taken to determine the stage of development. Dissection of the seeds was continued for 75 days. The data summarized in table 3 presents the minimum and maximum period of time necessary for the development of each of the four larval instars. The first record for the completion of the fourth larval instar occurred 46 days after hatching and the first adult did not appear for 67 days. These data differ greatly from those of Pinckney (1937), who reported the average length of

Table 3.—Number of days from hatching of egg to end of stage shown.

	Larval Instar				
	I	II	III	IV	Pupa
Minimum	13	25	35	46	67
Maximum		33	53	72	75+

the larval stage to be approximately 2 weeks in North Carolina, and from those of Randolph & Gillespie, who recorded in Texas, the first adults 22 days after the egg was laid. The difference is even more striking when the average 12-day incubation period is added to the developmental periods cited above.

Pupation. The first pupae was discovered in mid-August and the minimum time for development from hatching of the eggs to the pupal stage was 46 days. The earliest record of completion of the pupal stage was 67 days after hatching.

New Generation Adults. New generation adults first appeared during the first week of September in 1954. The maximum times for both the pupal and adult stages were not determined, owing to a shortage of tagged material at this late date.

The adults emerge from the seeds by forcing out a disk-shaped cap in the seed coat, which the last instar larvae prepare before pupation. The adults do not emerge until dehiscence of the pod, since they are unable to penetrate the dry pod. The newly emergent adults seek hibernating sites and were found to spend the winter in protected places, such as cracks in fence posts, under lichens and loose bark on trees, and in trash and weeds along fence rows. Grain storage bins and granaries provide the most common overwintering site, as considerable hairy vetch seed is present among the harvested grain. Many adults do not emerge from the seeds until the following spring.

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Free-choice Administration of Ronnel in a Mineral Mixture for the Control of Cattle Grubs[1]

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ABSTRACT

Technical ronnel incorporated in a 3:1 salt-bonemeal mixture was made available on a free-choice basis to cattle grub-infested calves for periods of 28 to 66 days. Outstanding control (100%) was achieved at concentrations of 48 and 40 grams ronnel per pound of mixture for 66 days. Inadequate control was obtained at lower levels (16 grams per pound) and over shorter periods (28 days). Six-fold variations in average total mineral consumption (0.31 to 1.88 oz. per day) were observed. Accompanying physiological events such as reduced erythrocyte cholinesterase activity (as low as 21% of pretreatment activity) and transient weight gain depression at higher levels of ingestion suggest that further research with this or other appropriate systemic chemicals be pursued before general recommendations for use be made.

Within the past few years a number of compounds have been developed that have a high degree of systemic effectiveness against cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.), as well as against a wide variety of other arthropod endo- and ectoparasites of livestock. These compounds have been administered orally, externally applied as sprays, and by parenteral injections. The choice of method of administration is partially dependent on the characteristics of the individual compound, and on the management practice most suitable for the individual livestock producer.

When a disease is of high morbidity and of unusual occurrence, the mode of administration of a medication may be quite difficult and still gain acceptance. Cattle grub infestations produce no high morbidity nor are they of unusual occurrence. Despite high total losses in the packing plant, the losses that accrue to the individual cattle producer are by no means obvious. When the cattle producer is managing a range rather than a feed-lot, his losses must be of sufficient magnitude to justify the time and expense of assembling cattle and providing individual treatment. Under such conditions self-treatment by the animals themselves would be highly desirable.

Self-treatment by cattle is obviously less precise than any method involving individual handling of stock. That such methods can become highly valuable additions to routine management practices is shown by the high degree of horn fly (*Siphona irritans* (L.)) and louse control obtainable with self-treatment rubbing devices (Rogoff & Moxon 1952, Hoffman 1954). Despite their limitations these, and a variety of similar devices, have become accepted in standard management practices because of their relatively high effectiveness coupled with extremely low cost of operation.

Current range practices in South Dakota do not involve extensive use of feed supplements in summer and fall, but are adaptable for the feeding of salt or salt-mineral mixtures. This paper describes attempts during the past 3 years to evaluate the possibilities of providing a systemic insecticide to cattle by allowing free-choice access to a low concentration of a candidate compound in a salt-mineral mixture. The candidate compound of choice when these experiments were initiated was ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl)phosphorothioate), also designated Dow ET-57. These experiments have served as a pilot study for self-treatment of cattle with systemic insecticides.

REVIEW OF LITERATURE.—Little has been reported previously on the use of mineral mixtures as carriers for systemic insecticides. Radeleff & Woodard (1956) discussed the problems involved in such oral administration. The early promise of phenothiazine in free-choice ingestion studies (Schwartz *et al.* 1954) was not confirmed by later work (Rogoff & Kohler 1956). Despite the limited work on mineral mixtures, a fair amount is known about continuous low-level feeding of systemic insecticides in feed supplements. Neither the work with feed supplements of Riedel *et al.* (1955) nor of Roberts *et al.* (1957) confirmed the earlier, apparently successful, use of phenothiazine.

The announcement of the high efficacy of ronnel as an oral drench (Lindquist 1956) opened the way for experiments with this material on a low-level, free-choice basis. Experiments with this insecticide were initiated shortly after it became available in 1956, by ourselves and by others. Preliminary reports of these experiments have appeared in scattered publications (Rogoff 1957, Rogoff *et al.* 1957). That portion of our work dealing with feed additives will be published separately.

Sturdy (1958a, 1958b) indicated high effectiveness with this compound when mixed with a protein supplement and fed to calves at 4.25, 8.5, and 17.0 mg./kg. daily for 8 months. No apparent toxicity to the animals was reported. French *et al.* (1958) showed more than 95% grub control with ronnel or Dowco 109 when administered as feed additives at the rate of 35 mg./kg. and 2.4 mg./kg., respectively, on each of 6 consecutive days. DeFoliart *et al.* (1958) also studied systemic insecticides in feed additives at high dosage levels for short periods. They reported 61 to 82% reductions in grub encystments by using Dowco 109 and ronnel.

MATERIALS AND METHODS.—In all the trials here reported ronnel was mixed thoroughly with a 3:1 salt-bonemeal combination. Three concentrations of ronnel were prepared, these being 16, 40, and 48 grams per pound of total mixture. The three formulae were: 16 gm. ronnel, 109.5 gm. bonemeal, 328.5 gm. salt; 40 gm. ronnel, 103.5 gm. bonemeal, 310.5 gm. salt; and 48 gm. ronnel, 101.5 gm. bonemeal, 304.5 gm. salt. The warmed, liquefied ronnel was added to warmed salt and thoroughly mixed. The

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cooled mixture was regranulated, screened, and mixed with the dry bonemeal. Packed in sacks or wide-mouthed jars the mixture retained good flowing characteristics during the time needed. Where consumption was recorded in check animals, the 3:1 salt-bonemeal combination was used alone.

Formulations were freshly prepared, never more than 1 month in advance of their being made available to the calves. The ronnel was obtained directly from the Dow Chemical Company. Stock salt (iodized) of various grades was used.

Calves originating in areas with a history of heavy grub infestation (*H. lineatum* and *H. bovis* in comparable numbers) were used as test animals. Tests were conducted in adjacent lots at Brookings in 1956, 1957 and 1958, and at Reed Ranch, a substation of the South Dakota Agricultural Experiment Station located in central South Dakota, in 1958. The test calves were individually weighed and lotted into uniform groups, by weight and sex except where specifically noted. The animals were housed in sheds divided into lots, each lot with an outside yard. Hay, grain, and water were provided in a comparable manner for each lot. Brome hay was offered free-choice. A mixture of equal parts oats and corn was offered to each lot on the basis of 4 pounds per calf per day.

Mineral boxes were located inside the sheds. The ronnel-salt-bonemeal mixture was provided for periods varying from 29 to 66 days, beginning in November or December of each year. Free-choice access to the mixture was permitted. This was the only source of mineral supplement during the test periods. At the end of each period the remaining mineral mixture was reweighed to determine the average consumption for each lot. The control lots were handled in the same manner except that ronnel was omitted from the salt-bonemeal mixture. The comparison of total mineral intake between the control and treated lots made it possible to evaluate the effect of ronnel on the palatability of the mixture.

Calculations of relative consumption are based on average weights of the calves at the start of each experiment. Average initial weight provides a maximum average intake figure. This figure is used arbitrarily since the length of treatment apparently exceeded the time necessary for maximum effect, and because consumption tended to decrease as the feeding periods continued. Total consumption is given in terms of average weights midway through the test periods.

All calves were individually weighed at monthly intervals. Symptoms of toxicity were watched for throughout the treatment periods. Grub extractions were made on all calves in lots at Brookings at 3 monthly intervals beginning close to the first of February in each year. At Reed Ranch grub palpations were made.

The cholinesterase determinations reported here have been made in laboratories of the South Dakota Agricultural Experiment Station, except where otherwise noted.

Blood in each instance was drawn from the jugular vein into a heparinized tube. The method of analysis was essentially that of Michel (1949). Duplicate analyses were run on each sample.

EXPERIMENTAL DATA.—The first exploratory work of this series of experiments was begun in the fall of 1956. Previous reports of the efficacy of ronnel applied as an oral drench at dosages of 100 mg./kg. (Lindquist 1956) gave slight indication of appropriate dosages for low-level, long-term applications. The initial plan for the 1956–57 experiments called for a continuous dosage of 10 mg./kg./day. Before work actually started, however, it was learned that R. A. Sturdy (personal communication) had successfully provided 20 mg./kg/day to calves for several months by way of feed, without any superficial indication of toxicity. With this information, a formulation was devised, using salt and bone meal, to provide an intended level of intake of 15 mg./kg./day on

Table 1.—Consumption, grub infestation, erythrocyte cholinesterase activity, and weight gain of calves allowed free-choice access to a ronnel-mineral mixture for 66 days. Brookings, S. Dak.

CRITERIA	TREATMENT (GRAMS RONNEL/LB. MIX)	
	48	0
Total calves (Dec. 17, 1956)	7	8
Consumption (per calf)		
Total mixture (oz./day)	0.62	—
Ronnel (mg.)		
Total (per kg., median)	525	—
Per day (per kg., initial)	9	—
Infestation		
Calves infested	0	8
Grubs/calf (range)	0	12–119
Grubs/calf (avg.)	0	78.3
Per cent reduction	100	—
Erythrocyte cholinesterase[a]		
Feb. 26, 1957 (40th day)		
Δ pH units/hour		
Range	0.06–0.34	0.58–1.22
Average	0.175**[b]	0.915
Activity (% control)	19	—
Weight gain (per calf)		
Initial weight (lbs.)	455	458
Daily gain (lbs.), 112 days	1.24	1.56
Per cent of initial weight	31	38

	TREATMENT (GRAMS RONNEL/LB. MIX)		
	48	16	0
Total calves (Dec. 23, 1957)	8	8	8
Consumption (per calf)			
Total mixture (oz./day)	0.54	0.37	0.59
Ronnel (mg.)			
Total (per kg., median)	536	121	0
Per day (per kg., initial)	8	2	0
Infestation			
Calves infested	0	4	6
Grubs/calf (range)	0	0–10	0–47
Grubs/calf (avg.)	0	2.4**	13.1
Per cent reduction	100	82	0
Erythrocyte cholinesterase			
Jan. 17, 1958 (25th day)			
Δ pH units/hour			
Range	0.06–0.31	0.19–0.46	0.30–0.49
Average	0.16**	0.32	0.39
Activity (% initial)	40	84	83
Feb. 14, 1958 (53rd day)			
Δ pH units/hour			
Range	0.09–0.24	0.25–0.54	0.32–0.55
Average	0.15	0.37	0.45
Activity (% initial)	37	97	96
Feb. 27, 1958 (69th day)			
Δ pH units/hour			
Range	0.10–0.34	0.30–0.66	0.42–0.59
Average	0.21**	0.42	0.52
Activity (% initial)	52	110	111
Weight gain (per calf)			
Initial weight (lbs.)	400	401	398
Daily gain (lbs.), 111 days	1.47	1.65	1.62
Per cent of initial weight	41	46	45

[a] Analyses by Dow Chemical Company.

[b] In this and following tables differences from check significant * at 5% level, ** at 1% level.

the assumption that calves would consume approximately 1 ounce of the total mixture per day.

As shown in table 1, actual average consumption during the 1956–57 tests was only 0.62 oz. per day, with a resultant dosage (calculated on the basis of average initial weight) of 9 mg./kg. The mixture was accessible for a 66-day period and the per calf consumption for the entire period was 525 mg./kg. (based on average weight midway through the treatment period).

The consumption figures for 1957–58 (table 1) for the same mixture (48 grams per pound) were comparable as were the figures for control animals having access to the ronnel-free salt-bonemeal mixture. Consumption of the lower level ronnel-mineral mixture, was, for no obvious reason, considerably less. Consumption among calves under similar conditions was considerably higher in 1958–59 (tables 2 and 3), but much lower among calves under different holding conditions (table 4). Thus consumption averages varied from 0.31 to 1.88 oz. per day in these tests; averages differing roughly by a factor of six.

The effectiveness of the ronnel-mineral mixture for controlling grubs in various tests is shown in tables 1 to 4. The outstanding success (100%) shown in 1956–57 at approximately 9 mg./kg./day for 66 days was repeated in 1957–58, but at 2 mg./kg./day of ronnel only fair control (82%) was recorded. The results in Brookings in 1958–59 are also outstanding at the higher dosage level (even for the shorter access period of 28 days) but distinctly mediocre for the five calves at the lower dosage for the full 59 days. The explanation of this relative failure is almost entirely ascribable to the one calf of this group having 58 grubs. Since this was a free-choice procedure, the assumption is that this one animal did not

Table 2.—Consumption, grub infestation, erythrocyte cholinesterase activity, and weight gain of calves allowed free-choice access to ronnel-mineral mixtures for varying periods. Brookings, S. Dak.

	Treatment (Grams Ronnel/Lb. Mix)				
	First 28 Days[a] (11/25–12/23)		Full 59 Days (11/25–1/23)		Control
Criteria	40	16	40	16	0
Total calves (Nov. 25, 1958)	6	6	5	5	11
Consumption (per calf)					
Total mixture (oz./day)					
First 28 days	0.91	1.30	0.91	1.30	1.88[b]
Last 31 days	0.56[b]	0.56[b]	0.46	0.39	1.40[b]
Ronnel (mg.)					
Total (per kg., median)					
First 28 days	350	185	350	185	0
Full 59 days	—	—	534	249	0
Per day (per kg., initial)					
First 28 days	12.8	7.0	12.8	7.0	0
Full 59 days	—	—	9.5	4.7	0
Infestation					
Calves infested	0	0	0	0	11
Grubs/calf (range)	0	0	0	2–58	6–57
Grubs/calf (average)	0	0	0	12	23.0
Per cent reduction	100	100	100	48	0
Erythrocyte cholinesterase					
Dec. 23, 1958 (28th day)					
Δ pH units/hour					
Range	0.04–0.20	0.08–0.36	0.04–0.36	0.07–0.42	0.36–0.46
Average[c]	0.09**	0.15**	0.09**	0.24**	0.40
Activity (% initial)	21	39	18	55	89
Jan. 23, 1959 (59th day)					
Δ pH units/hour					
Range	0.23–0.30	0.19–0.33	0.05–0.22	0.20–0.61	0.40–0.75
Average	0.28	0.24	0.15	0.38	0.50
Activity (% initial)	61	61	31	84	111
Feb. 23, 1959 (90th day)					
Δ pH units/hour					
Range	0.25–0.33	0.18–0.31	0.15–0.30	0.22–0.37	0.26–0.46
Average	0.29	0.24	0.22	0.30	0.34
Activity (% initial)	64	61	46	68	75
Weight gain (per calf)					
Initial weight (lbs.)	394	410	419	400	402
Daily gain (lbs.), 134 days	0.84	1.30	1.12	1.37	1.21
Per cent of initial weight	29	43	36	46	40

[a] Since 28 day and 59 day animals were together for first 28 days, an alternate summation of the 28-day data is given in table 3.
[b] Salt-bonemeal alone, no ronnel.
[c] Significance between ronnel treatments at 5% level.

Table 3.—Consumption, grub infestation, and erythrocyte cholinesterase activity of calves allowed free-choice access to ronnel-mineral mixtures for 28 days.[a]

Criteria	Treatment (Grams Ronnel/Lb. Mix)		
	40	16	0
Total calves (Nov. 25, 1958)	11	11	11
Consumption (per calf)			
Total mixture (oz./day)	0.91	1.30	1.88
Ronnel (mg.)			
Total (per kg., median)	341	192	0
Per day (per kg., initial)	12.8	7.0	0
Infestation			
Calves infested	0	2	11
Grubs/calf (range)	0	2–58	6–57
Grubs/calf (average)	0	5.5	23.8
Per cent reduction	100	77	0
Erythrocyte cholinesterase			
Dec. 23, 1958 (28th day)			
Δ pH units/hour			
Range	0.04–0.20	0.05–0.42	0.36–0.46
Average	0.09	0.19	0.40
Activity (% initial)	19	46	89

[a] Data drawn from all calves included in table 2.

Table 4.—Consumption, grub infestation, and weight gain of calves allowed free-choice access to ronnel-mineral mixtures for 29 days, Reed Ranch, S. Dak.

Criteria	Treatment (Grams Ronnel/Lb. Mix)		
	40	16	0
Total calves (Nov. 20, 1958)	21	11	15
Sex	heifers	steers	bulls
Consumption (per calf)			
Total mixture (oz./day)	0.47	0.31	0.67
Ronnel (mg.)			
Total (per kg., median)	164	46	0
Per day (per kg., initial)	6.4	1.7	0
Infestation			
Calves infested	9	9	15
Grubs/calf (range)	0–67	0–27	8–67
Grubs/calf (average)	10.0**	9.5**	28.7
Per cent reduction	65.2	66.9	0
Weight gain (lbs./calf)			
Initial weight	412	389	419
Daily gain, 102 days	1.69	1.94	2.15
Correction for sex[a]	0	−0.2	−0.45
Corrected daily gain	1.69	1.74	1.70
Per cent of initial weight (meas.)	66	81	83
Per cent of initial weight (adj.)[a]	66	72	66

[a] Correction based on local animal husbandry experience with similar rations and similar breeding.

consume its appropriate share of ronnel. Consideration of all data for the first 28 days (table 3) reduces the influence of the one heavily infested animal, but here again is an explanation of the lesser control obtained.

Only mediocre control (about 65%) was obtained in the Reed Ranch 1958–59 tests (table 4). However these tests ran only 29 days and total consumption was relatively low. Again variable consumption is regarded as the explanation of these results.

Both *H. lineatum* and *H. bovis* were present in all tests in such numbers as to indicate no differential susceptibility to treatment.

Erythrocyte cholinesterase studies in connection with the 1956–57 data consisted only of a comparison of activity in the treated and untreated animals after 40 days of treatment. These data (table 1) show that all treated animals had considerably lower activity than the controls. The cholinesterase activity of the treated animals was only 19% as great as that of the controls.

Cholinesterase studies the following year (1957–58, table 1) were more complete and are presented in terms of pretreatment activity of the individual animals. The degree of depression was less severe than during the previous year (at the same level of treatment) but still was greater than 50%. It is noteworthy that at the lower dosage of 2 mg./kg./day no depression was noted at either the 25th, the 53rd, or the 69th day following the initiation of treatment.

Significant erythrocyte cholinesterase depression was again noted in the 1958–59, Brookings tests (tables 2 and 3), especially for the higher level dosage. Examination of the cholinesterase levels of the one heavily infested animal on the 16-gram-per-pound mixture revealed no significant depression (83% activity at 28 days compared with an average of 89% activity among the controls, and 100% pretreatment activity at 59 days) thus supporting the assumption that this animal had not been consuming its proportionate share of the ronnel-mineral mixture.

Weight gain studies throughout these experiments (tables 1, 2, 4), reveal no advantage in this connection related to treatment. Where severe cholinesterase depression was observed, as in tables 1 (48 grams per pound data), and 2 (40 grams per pound data), deficiencies in weight gains were also observed.

Throughout these tests the animals were closely observed for signs of gross toxicity. Of the 77 calves exposed to ronnel-mineral mixtures only two showed any signs of sickness. The first of these, in 1956, was almost certainly not related to treatment. Though its cholinesterase level was very low on the 40th day of treatment (Δ pH units per hour 0.064) another calf in apparently excellent health had activity almost as low (Δ pH units per hour 0.083). Other animals in other years have had as low or even lower activity levels without showing signs of illness (see tables 1 and 2). The critical information, however, is that this calf had a history of coccidiosis (oocysts were found) and in the 1-month period before treatment was started this calf actually lost 3 pounds in weight.

The second sick calf was observed among the 1958–59 Brookings calves after 17 days' access to the 40-grams-per-pound ronnel-mineral mixture. Cholinesterase activity in the red blood cells of this calf was low (ΔpH units per hour 0.07, about 15% of pretreatment activity) but not as low as three other animals in the same group, 28 days posttreatment. This animal, however, showed symptoms related to organophosphate poisoning and responded well to removal of the ronnel mixture, with recovery in a matter of several weeks. No grubs were later found in this calf.

DISCUSSION.—The data presented demonstrate the possibility of excellent control of cattle grubs by free-choice administration of ronnel in a salt-bonemeal mixture. The lower levels at which control becomes marginal were also demonstrated. The success shown is of a high enough order to warrant further investigation of this technique of administration.

The success of grub control was somewhat marred by concurrent physiological events that might be regarded as undesirable. At those levels of administration that proved most successful in eliminating grubs, erythrocyte

cholinesterase levels were significantly depressed, and weight gains were somewhat reduced.

Neither of the negative effects just mentioned, however, can be regarded as definitely disturbing. The cholinesterase depression in the red blood cells may or may not be associated with depletion of this enzyme in more critical tissues. Such depression in the erythrocytes, when accomplished at a slow enough rate, has not been correlated with deleterious physiological events (Radeleff & Woodard 1957). Erythrocyte cholinesterase activity depression, however, potentially places the animal in jeopardy in the event of further rapid depletion of this enzyme by other physiological events, whether induced by organophosphate insecticides or not.

The apparent reduction in weight gains in those animals maintained at high treatment levels is difficult to evaluate. Following the close of treatment these differences tended to disappear. Furthermore statistical studies of the data did not reveal significance approaching the 5% level.

Unequal dosage due to variable mineral consumption by different animals appears to be a major disadvantage of this method of treatment. The six-fold variation in mineral consumption here reported may be due to previous management and state of nutrition of the animals. The amount of ronnel in the mineral mixture did not appear to have a direct bearing on total consumption (compare tables 1, 2, and 4).

The present paper calls attention to the promise displayed by the free-choice, low-level administration of a systemic agent in a mineral mixture under dry-lot contitions. The choice of ronnel as the systemic agent was dictated by its availability. It would appear desirable to extend the present investigation in several directions. Further work can be done with ronnel by varying the conditions of intake, possibly by reducing the concentration and extending the access-period to smooth out variability of consumption. Studies under pasture or range conditions, and at various times of the year would be desirable. The addition of a nutrient or some other additive to control salt intake might be profitable. Obviously another line of investigation is to explore the many other promising systemics, with the possibility of more uniform intake or less obvious physiological disturbance of the host. Reduced physiological interference would be especially desirable when treating young calves, pregnant cows, or animals under any other metabolic stress.

Other directions of research might include investigations on formulation, including palatability, stability and packaging, other physiological influences in addition to those affecting weight gain and erythrocyte cholinesterase, and concurrent effectiveness against other internal and external parasites.

The possibility of inducing insecticide resistance cannot be ignored. If the deficiencies of this procedure involve incomplete control of grubs exposed to the chemical, the problem of resistance could become significant. However, if, as appears at least partially to be the case, the failures are caused by irregular consumption by the host (and hence a failure to significantly expose individual grubs to insecticide) a selection differential within the grub population would be less involved.

From a management point of view, the simplicity of the free-choice mineral mixture procedure would make this technique an especially valuable one under conditions of high labor cost, though at current prices the increased quantity of ronnel required for this treatment may easily offset the reduction in labor costs. In large scale eradication campaigns a more positive dosage method (whether bolus, spray, or injection) might be desirable, but in long-term population control ventures the labor-saving mineral procedure might become the method of choice.

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Chemical Control of the Carrot Rust Fly, *Psila rosae* (F.), in Western Washington[1]

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ABSTRACT

The carrot rust fly (*Psila rosae* (F.)) is normally controlled in Washington by soil and surface applications of chlorinated hydrocarbons. In 1957 a strain of *Psila rosae* highly resistant to the chlorinated hydrocarbons appeared in the Lake Sammamish region in northwestern Washington. The carrot crop was destroyed despite repeated soil and surface treatments with chlorinated hydrocarbons. Twenty-two compounds, including chlorinated hydrocarbons and organic phosphates, were tested as soil or furrow treatments. Am. Cyanamid 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate), dicapthon, and parathion gave fair control when used as furrow treatments. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate gave outstanding control as a furrow treatment when used at rates of 1, 2, 3 or 4 lbs. of active ingredient per acre. Diazinon was ineffective at 5 pounds per acre when used as a total soil treatment. All other compounds tested were ineffective when used as soil or furrow treatments.

The carrot rust fly (*Psila rosae* (F.)) has been a pest of carrots, parsnips, celery, and parsley in Washington for many years. First records of this insect in the state were by A. L. Melander who collected specimens at Nooksack in Whatcom county in 1908 and at Olga in San Juan county in 1910. Hanson & Webster (1941) reported that the rust fly was a serious pest in the vegetable growing areas near Tacoma and Seattle in 1928. Glasgow (1929, 1931) first used naphthalene flakes in controlling this pest. Until the chlorinated hydrocarbons became available, crude naphthalene flakes was the only satisfactory insecticide used by growers in Washington.

In western Washington there are either three complete generations, or two and a partial third brood a year. The first generation of adults generally emerges from May 1 to May 20. The second generation normally emerges from July 15 to about September 1. The third generation which starts in September, overlaps with the second and continues until the advent of cold weather.

Damage from the carrot rust fly is sporadic rather than uniform or contiguous throughout Washington. At present, chlorinated hydrocarbons used as total soil or surface treatments, are successfully used to control this pest everywhere except in the Lake Sammamish region. Previously these methods and material gave excellent control of the carrot rust fly in this area. Approximately 500 acres of carrots, mostly for fresh market produce, are normally grown in this region. It is regarded as the most important carrot-growing area in the state. The soil is classified as Puget silty clay loam. These carrots are planted at regular intervals from March until August to supply the demand for fresh market produce. In 1957, damage from the rust fly caused the carrot crop to be a total loss despite soil and multiple surface treatments with chlorinated hydrocarbons. This paper deals with tests conducted in western Washington from 1953 to 1958, during which interval successful control measures were developed using chlorinated hydrocarbons, or organic phosphates when the chlorinated hydrocarbons failed.

METHODS—Except for the 1958 tests, five replications of single rows in a randomized block design were used in all experiments. A replication consisted of 80 lineal feet in the 1954–55 work and 20 lineal feet in the 1956–57 tests. Evaluation of compounds was made by grading plants as clean or infested. In the 1954 and 1955 work, 50 plants per plot or 250 per treatment were selected at random and classified for injury. All plants in the plots were examined in the 1957 and 1958 experiments. Plants were classified as infested if there was any evidence of rust fly damage. The work in 1954 and 1955 was conducted at American Lake. These tests were carried out in soil classified as Spanaway gravelly sandy loam. The tests in 1957 and 1958 were conducted in the Lake Sammamish area in soil classified as Puget silty clay loam.

Tests in 1954.—The toxicants were used at dosages lower than rates calculated to give economic control to facilitate evaluation of their effectiveness against the carrot rust fly. All compounds were applied as surface treatments. When the insecticide was applied in a single application it was applied when the foliage reached the first true leaf stage. The carrot normally attains this growth about a week after emergence. The parsnips arrive at this stage of growth a few days later. When two surface applications were employed, one-half the dosage was applied at the first true leaf stage and the remainder 3 weeks later.

Tests in 1955.—Treatments were applied in a single application as a soil or surface treatment. For the soil treatments, the insecticides were applied in a 2-foot band and then worked into the soil 5 or 6 inches deep using a 24-inch Rototiller. The rows were smoothed and packed, then seeded down the middle with a Planet Jr. seeder. The surface applications were sprayed in a 1-foot band over the rows using a 3-gallon hand sprayer when the plants reached the first true leaf stage.

Tests in 1957.—Treatments were applied as total soil or furrow treatments. Emulsion concentrates were used in the soil treatments. The correct amount of toxicant for each 20-foot row was mixed with 3 quarts of water and sprinkled from a watering can in a 2-foot band. The materials were worked into the soil and the rows planted

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in the manner described for the 1955 tests. A Gandy chemical attachment mounted on a motor driven Planet Jr. seeder was used to apply the granules used in the furrow treatments.

Tests in 1958.—All insecticides were applied as furrow treatments. The granulated materials were applied using the methods described for the 1957 furrow treatments. When dusts or wettable powers were used, a multiple gear seeder was used to place the chemical and seed in the furrow simultaneously. The amount of seed for each 20-foot of row, using the multiple gear seeder, was obtained by raising the front wheel of the Planet Jr. seeder and turning it until it had traversed 20 lineal feet. Using this method it was anticipated that uniform seeding rates would be obtained with the Planet Jr. and the rod-row multiple gear seeders. A simple split-plot design, using single row plots, was employed. There were four replications. To facilitate evaluation of the large number of carrots examined in this work, commercial methods of preparing vegetables for retail market were used. The carrots were washed, scrubbed, and cleaned mechanically, then moved along a brightly lighted conveyer belt where they were classified as clean or infested.

Table 1.—Percentage of carrots or parsnips showing rust fly injury after treatment with surface applications of insecticides. American Lake, Washington, 1954.

Insecticide	No. of Applications	Total Active Ingredient /Acre(Lb.)	Plants Infested (%)	Analysis[a]
Test No. 1—Carrots planted May 23, harvested Aug. 25				
Heptachlor	1	0.3	8	a
Dieldrin	2	.6	12	b
Heptachlor	2	.6	13	bc
Endrin	2	.6	14	bcd
Dieldrin	1	.3	15	bcd
Chlordane	1	.6	17	cd
Chlordane	2	1.3	17	cd
Aldrin	1	.3	18	d
Endrin	1	.3	22	e
Aldrin	2	.6	24	e
Isodrin	2	.6	25	e
Toxaphene	2	1.6	25	e
Isodrin	1	.3	32	f
Toxaphene	1	.6	38	g
Untreated	—	—	84	h
Test No. 2—Carrots planted June 7, harvested Sept. 23				
Heptachlor	2	0.6	12	a
Heptachlor	1	.3	14	a
Dieldrin	1	.3	14	a
Chlordane	1	.6	15	a
Endrin	2	.6	16	ab
Dieldrin	1	.6	16	ab
Aldrin	1	.6	16	ab
Aldrin	2	.6	17	ab
Endrin	1	.3	17	ab
Chlordane	2	1.3	20	bc
Aldrin	1	.3	21	bc
Isodrin	2	.6	21	bc
Toxaphene	2	1.6	21	c
Isodrin	1	.3	23	c
Toxaphene	1	.6	23	c
Untreated	—	—	64	d
Test No. 3—Parsnips planted May 23, harvested Sept. 2				
Heptachlor	1	0.6	10	a
Dieldrin	1	.3	10	ab
Heptachlor	2	.6	11	abc
Dieldrin	2	.6	11	abc
Endrin	2	.6	13	bc
Aldrin	2	.6	14	c
Heptachlor	1	.3	18	c
Endrin	1	.3	28	e
Aldrin	1	.3	29	e
Isodrin	2	.6	39	f
Isodrin	1	.3	45	g
Untreated	—	—	85	h

[a] Duncan's Multiple Range Test—if two treatments do not have a common letter for a given column, then the treatments are significantly different at the 5% level. Conversely, if two treatments in a given column have a common letter then they are not significantly different.

Table 2.—Percentage infestation by carrot rust fly in carrots treated with insecticides applied as soil or surface treatments at 2 pounds actual ingredient per acre. American Lake, Washington, 1955.

Treatment	Method of Application	Per Cent of Plants Infested
Dieldrin dust	soil	3
Dieldrin EC	surface	5
Aldrin WP	soil	6
Dieldrin WP	surface	6
Dieldrin dust	surface	6
Heptachlor EC	soil	6
Heptachlor WP	surface	6
Aldrin WP	surface	7
Heptachlor WP	soil	7
Heptachlor dust	soil	7
Aldrin dust	surface	7
Untreated	—	8
Heptachlor dust	surface	8
Untreated	—	9
Aldrin EC	soil	9
Aldrin dust	soil	10
Aldrin EC	surface	10
Dieldrin EC	soil	10
Dieldrin WP	soil	10
Heptachlor EC	surface	14

RESULTS.—(See tables 1 to 5.)—The results of three individual experiments are presented in table 1. This work was conducted at approximately the same time in the same field under similar conditions. Since a total of 60 treatments were involved in table 5, three checks, each consisting of five replications, were included. The results are listed separately to show the irregularity of infestation typical of this pest.

DISCUSSION.—In 1954, toxaphene and isodrin were the

Table 3.—Percentage infestation by carrot rust fly in carrots harvested after soil and furrow applications of insecticides. Lake Sammamish, Washington, 1957.

Insecticide	Method of Application	Pounds per Acre[a]	No. of Carrots Examined	Per Cent of Plants Infested
Untreated	—	—	487	100
Dieldrin EC	soil	4	435	100
Endrin granules	furrow	5	482	100
Heptachlor EC	soil	2	466	100
Heptachlor EC	soil	4	418	99
Aldrin granules	furrow	8	415	100
Di-Syston granules	furrow	2	502	89
Parathion granules	furrow	4	510	56
Thimet granules	furrow	5	537	20

[a] Rates for furrow treatments based on the number of lineal feet in an acre with rows 15 inches apart.

Table 4.—Plant emergence, number of carrots examined, percentage infestation, and weight of marketable carrots after treatments with insecticides applied to the soil at 5 pounds of active ingredient per acre. Lake Sammamish, Washington, 1958.

Insecticide	Plant Emergence	No. Carrots Examined	Pounds of Marketable Carrots[a]	Per Cent of Plants Infested
1 Thimet	465	422	78	57
2 Diazinon	646	526	43	64
3 V-C 13[b]	627	595	42	67
4 Ethion	652	435	27	72
5 Co-Ral[c]	549	495	12	75
6 Kepone	739	556	15	75
7 UCC 8305	656	542	30	75
8 Heptachlor	660	614	19	76
9 Trithion	784	548	31	77
10 Thiodan	506	410	11	81
11 Aldrin	585	483	12	82
12 Delnav[d]	657	484	12	83
13 Sevin	603	417	19	87
14 Toxaphene	612	440	3	90
15 Phosphamidon	617	458	6	90
16 Check	662	451	3	94
17 Perthane[e]	704	435	0	99
18 Malathion	831	422	0	99

[a] Sound carrots 3 inches or more in length without distortion.
[b] *O*-2,4-dichlorophenyl *O,O*-diethyl phopshorothioate.
[c] *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phopshorothioate.
[d] 2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate).
[e] 1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane.

least effective of the insecticides tested. There was no significant difference between heptachlor, dieldrin, aldrin, endrin, or chlordane, although heptachlor consistently had the lowest infestations. Two applications gave no better control than a single one. With two applications, one apparently was wasted since the treatment did not coincide with the appearance of the rust fly. Timing appeared more important than dosage because there was no significant increase in control when the dosage of surface applications was increased.

The 1955 infestations were too low to permit evaluating different formulations of aldrin, dieldrin and heptachlor used as soil or surface treatments.

Information concerning infestations in the Lake Sammamish region was not obtained until the first crop had been destroyed; therefore, the 1957 experiments were not established until late July. The field in which the work was conducted had been treated earlier in the season with heptachlor at 4 lbs. actual per acre. The carrots in these trials were subjected to extremely heavy populations since they were surrounded by fields of carrots destroyed by rust fly. The roots of all carrots except those in the parathion and Thimet® (*O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate) treatments were destroyed after germination. However, the tops generally remained green so that harvest counts could be made. The carrots in the Thimet and parathion treatments grew to normal size even though some were heavily infested with rust fly larvae. It appeared that these two treatments had enough residual action to allow the carrots to develop to normal size while the others were initially ineffective or had limited residual action.

No insecticide tested in 1958 gave control as a total soil treatment. The percentage of infestation in the carrots

Table 5.—Plant emergence, weight of marketable carrots, numbers of sound carrots, and percentage damaged by rust fly after application of insecticides as furrow treatments. Lake Sammamish, Washington, 1958.

Insecticide[a]	Active Ingredient (Lb./A.)	Plant Emergence	Pounds of Marketable Carrots[b]	Plants Infested (%)	Sound Carrots Harvested	
					Total No.	Analysis[c]
Check	—	1,957	0	100	12	a
Co-Ral WP	1	2,560	0	98	12	a
UCC 8305 WP	1	1,402	2	97	26	a
Thiodan	1	1,529	2	97	27	a
Aldrin	3	1,482	1	95	35	a
Kepone WP	1	2,434	1	92	42	ab
Kepone WP	2	2,725	1	92	47	ab
Sevin	1	858	5	94	52	ab
Kepone	3	2,526	1	92	74	ab
Phosphamidon	1	1,480	2	85	104	ab
Ronnel	1	1,138	11	88	116	ab
Sevin	4	2,304	20	83	125	abc
Ethion	1	1,770	6	86	132	abc
Phosphamidon	2	1,826	10	96	134	abc
Check	—	1,601	9	86	141	abc
Heptachlor	5	2,213	6	85	163	abc
Phosphamidon	4	1,724	9	80	168	abc
Thiodan	4	1,593	22	79	247	abcd
Co-Ral WP	2	2,391	10	79	250	abcd
Thiodan	3	1,647	26	79	260	abcd
Co-Ral WP	3	2,775	13	79	265	abcd
Ronnel	4	1,199	38	68	275	abcd
Thimet	3	855	94	27	290	abcde
DDT	5	1,555	40	73	310	abcdef
Thiodan	2	1,864	34	69	323	abcdef
Ronnel	3	1,775	47	64	332	abcdef
Sevin	3	1,451	75	60	334	abcdef
Sevin	2	1,177	58	66	339	abcdef
Malathion	5	1,875	52	70	376	abcdefg
Check	—	1,616	14	67	380	abcdefg
Phosphamidon	3	1,658	52	65	399	abcdefg
Trithion	1	1,731	66	55	446	bcdefgh
Dipterex	5	1,064	39	70	522	cdefghi
Thimet	1	364	133	30	575	defghij
Ethion	2	1,872	66	54	579	defghijk
Ronnel	2	1,642	78	48	615	defghijk
UCC 8305 WP	3	2,623	63	51	643	defghijkl
Trithion	3	1,853	130	40	673	efghijkl
Guthion	1	1,461	80	44	695	fghijkl
Thimet	2	897	164	16	750	ghijkl
Ethion	3	1,959	89	44	753	ghijkl
Trithion	4	1,937	102	46	757	ghijkl
Guthion	3	1,737	138	32	766	ghijkl
Parathion	3	986	199	10	791	hijkl
Parathion-DDT[d]	3	1,147	179	13	837	ijklm
UCC 8305 WP	2	1,885	91	34	844	ijklm
Guthion	2	1,620	109	37	879	ijklm
Ethion	4	1,705	150	30	889	ijklm
Dicapthon dust	1	2,121	71	41	957	jklmn
Trithion	2	1,541	128	27	999	klmn
Guthion	4	1,448	138	28	1,026	lmn
Am. Cyanamid 18133	3	2,552	176	18	1,202	mno
Am. Cyanamid 18133	1	1,958	120	25	1,210	mno
Dicapthon dust	3	2,851	185	19	1,311	nop
Am. Cyanamid 18133	2	1,964	160	15	1,438	opq
Dicapthon dust	2	2,623	154	16	1,469	opq
Diazinon	2	2,028	260	3	1,585	pq
Diazinon	1	2,013	204	9	1,593	pq
Diazinon	3	2,607	284	2	1,715	q
Diazinon	4	2,680	267	2	1,723	q

[a] Granules unless otherwise noted.
[b] Sound carrots 3 inches or more in length without distortion.
[c] See footnote a, table 1.
[d] 1.5 lbs. each of parathion and DDT applied.

harvested in 1958 should be considered conservative, particularly for the ineffective treatments, since some plants were killed shortly after emergence and these were not recorded.

Despite precautions to insure equal seeding rates, seeding with the rod-row multiple gear seeder appeared somewhat heavier than with the Planet Jr. seeder. Since some treatments were quite phytotoxic, both the number of sound carrots and the percentage infestation should be considered in evaluating treatments. Because the plants were too crowded, some died from causes other than rust fly. For this reason then, the percentage of control could not be considered as the dividend obtained by dividing the number of sound carrots by the emergence count.

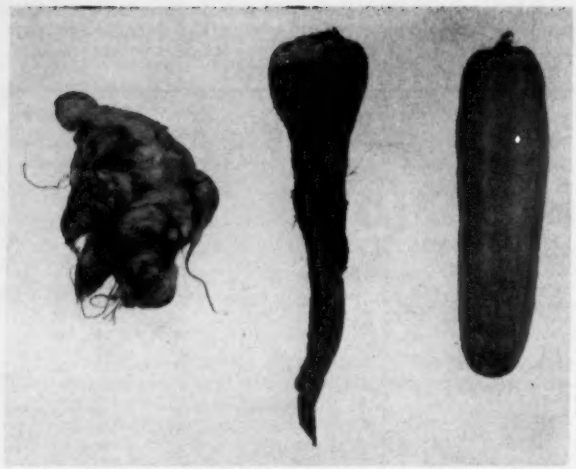

FIG. 1.—Normal carrot on right from Diazinon-furrow treatments. Distorted and infested carrots, shown at center and left, are carrots found in untreated and treated plots other than Diazinon-furrow treatments.

When applied as furrow treatments, Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate), UCC 8305 (P-chloro-2,4-dioxa-5-methyl-P-thiono-3-phosphabicyclo (4.4.0) decane), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), aldrin, Kepone® (1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo [5.2.1.0^{2,6}.0^{3,9},.0^{5,8}] decan-4-one.), Sevin® (1-naphthyl N-methyl carbamate), phosphamidon, ronnel, heptachlor, DDT, malathion, Trithion® (S-(p-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), ethion and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3,-benzotriazine-3-methyl) phosphorodithioate) were ineffective at all rates tested. Thimet, dicapthon and Am. Cyanamid 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) gave fair control. Thimet was so phytotoxic that relatively few plants germinated. Since the seeds were overplanted, the surviving carrots in the Thimet treatment grew large and were responsible for the relatively high weights for this treatment. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate was superior at all rates tested, and was the only treatment that gave economic control. The degree of control obtained with this toxicant was remarkable, considering the severity of the infestation. Carrots in the untreated and ineffective treatments were distorted and heavily infested. (See fig. 1.)

The question as to why rust flies in this particular area were no longer controlled by chlorinated hydrocarbons arose. Perhaps the flies, having a limited flight range, were confined to the valley and subjected to continuous treatments of chlorinated hydrocarbons for a number of years. It would be expected that resistance to chlorinated hydrocarbons could develop at an accelerated rate in areas restricted to carrot production and subjected to repeated applications of chlorinated hydrocarbons.

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Lepidoptera Associated with Grasses Grown for Seed in Eastern Washington[1]

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ABSTRACT

Various sampling methods, especially the use of light and field-emergence traps, combined with literature references to observed damage, provide estimates of the abundance and importance of the species concerned. Thirty-four species, representing four lepidopterous families, are listed in tabular form. *Crambus topiarius* Zell., *Crymodes devastator* (Brace), and *Chionodes psiloptera* (Busck) were found particularly destructive, and these, together with three other important species, are separately discussed. Temperature, humidity, and precipitation data were used in studying the relationship of climatic factors to light-trap catches of adults.

Information in the following discussion was gained mostly from a survey of the insects affecting grasses grown for seed between Spokane, Washington, and the Idaho border. The study was initiated because damage to grass crops, particularly bluegrasses and fescues, has been increasing.

Subterranean lepidopterous larvae attacking the host plant at or near the crown have been found to be the most obvious pests. Injurious species have been found in the families Noctuidae, Crambidae, Gelechiidae, and Arctiidae.

Investigation, which continues, involved the use of light traps, weather observations, field emergence and laboratory emergence traps on seven varieties of grass, and soil sampling for larvae. Light traps[3] were used to

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[2] Graduate Research Assistant and Assistant Entomologist, respectively. The writers are indebted to Dr. W. C. Cook, Entomologist at the U. S. Department of Agriculture Truck Crop Insect Laboratory at Walla Walla, Washington, and to specialists at the U. S. National Museum, for making initial identifications of most of the adult Lepidoptera discussed.

[3] Manufactured by Gardener Manufacturing Company, Horicon, Wisconsin. The attractant source is a General Electric 15-watt black-light tube.

trap most of the adults. The number of moths caught with light varies with the species (Cook 1928); thus the presence of several individuals of a troublesome species does not necessarily indicate its local abundance. Observation of a species in a field emergence trap (over grass), as well as its known record of injury, is considered sufficient evidence to class it as a suspected grass feeder. If a reasonably large number of individuals of a particular species is found in both the emergence and the light traps, its significance as a local pest is assumed.

Weather observations have occupied two main sites. One was the Spokane River valley, an area of irrigated agriculture averaging 2,050 feet elevation; the other was in the foothills, some 350 feet higher and directly to the north, consisting of mixed coniferous forest and nonirrigated agriculture. At each site, only a few feet from one of the two light traps, are situated (in the summer) a Foxboro Class 1 hygrothermograph, in an instrument shelter, and a rain gauge. This arrangement provided continuous data on temperature, relative humidity, and rainfall from the immediate vicinity of the light traps in two representative grass-growing areas.

Most of the information in table 1 and the discussion which follows are recorded from investigations conducted during 1957. The list of species comprises all Lepidoptera that are assumed to be grass feeders captured in light traps. Citations of literature that state that a species feeds on grass, as well as emergence-trap records where such exist, are also presented.

Various larvae of the Crambidae and Noctuidae are well-known pests of grasses. The present study, as well as that of Bohart (1947), appears to substantiate this in the Western United States. In a survey of California lawn pests, Bohart found that *Crambus bonifatellus* (Hulst) and *C. sperryellus* Klots were particularly damaging. The former is little known, and the latter is not known from the grass fields near Spokane. A pyralid, *Nomophila noctuella* (D. & S), the lucerne moth, was considered to be a contributor to lawn damage in California. Identification of light-trap catches near grass fields in Washington for 1958 show large numbers of this moth, but investigation of its bionomics in such areas is yet to be initiated.

Bohart records three noctuid species which occasionally damage lawns severely in California. These are the armyworm, *Pseudaletia unipuncta* (Haw.), the granulate cutworm, *Feltia subterranea* (F.), and the variegated cutworm, *Peridroma margaritosa* (Haw.). These have not been found associated with grasses in the present study, nor has the infrequent pest *Hylephila phylaeus* Drury, a hesperiid.

The occurrence of full moon was associated with definite decreases in the number of most of the destructive species of adult Lepidoptera captured at light traps. This relationship was noted by Williams (1936), and has been commented on further by Cook in correspondence.

DISCUSSION OF SIGNIFICANT SPECIES.—*Chionodes psiloptera* (Busck).—This small, dark moth, the only gelechiid of any consequence caught by light trap, is a grass pest with severe destructive potential in certain localized areas. Initial identification by Dr. J. F. Gates Clark, of the U. S. National Museum, indicated that this moth has been collected previously only in Ontario, Canada. The plant host was then unknown. Its flight is characteristically low, irregular, and of short duration in the daytime. Larvae tunnel into the crown and root systems, where their feeding places are partly covered by loose webbing.

Adults were collected in greater numbers than those of any other species during a 24-hour period. Their appearance has been limited almost entirely to portions of the foothills. Flight activity in 1957 was between the middle of April and the middle of May. Nearly all specimens were caught just following heavy rains. When they were captured the relative humidity was low, the temperature rising, and the moon was new.

Crambus topiarius Zell.—Larval collections in the field, as well as adult field- and laboratory-emergence records, indicate that this well-known pest of grasses is probably the most abundant species of *Crambus* in the Spokane area. Many were caught in the foothills light trap, though the moth is also widespread in the valley. The first of two peak light-trap catches coincided with the large catch of *Chionodes;* the second, also in the foothills trap, came toward the end of June, late in a flight season that had begun about a month earlier.

These two peaks, associated with rising temperatures, low relative humidities, lack of moonlight, and periods following heavy rains, may indicate that the species overwinters as mature larvae or as prepupae. Both feeding and nonfeeding larvae were found in the spring, which is further evidence of this overwintering. Adults of the latter larvae emerged in the laboratory when the first peak flight occurred in the field.

Crambus dorsipunctellus Kft.—No mention of the bionomics of this crambid has been seen in the literature. Only one specimen was trapped upon emergence from a field, but many field observations and light-trap captures suggest its importance as a grass feeder. Periods of large catches, which occurred especially in the foothills light trap, coincided with the ending of rains as well as with warm temperatures.

Crambus plumbifimbriellus Dyar.—Two generations of this relatively little-known moth may occur each year near Spokane. Several field emergences and many observations show that it is a grass pest. The heaviest catches, possibly a second generation, directly followed early August rains in the valley during a warm period.

Crambus trisectus (Wlk.).—The history of destruction of Midwest grasses, oats, and corn by *Crambus trisectus* (Ainslie 1927) makes its appearance in Northwest grass fields one to be noted. The 1957 captures were mostly from the valley light trap in late summer. Climatic variation did not appear to influence a steadily distributed series of catches.

Crymodes devastator (Brace).—Light-trap catches of the widely known glassy cutworm moth did not indicate adequately its importance as a grass pest in the areas under survey. Some authorities consider it the most serious pest of its kind to corn and other grasses. In many of the fields examined in this investigation, both in the foothills and in the valley, the larvae were found in large numbers and were very destructive. A single generation appears to occur annually. The period of adult capture extended from mid-July to the end of August. Rains in August coincided with the largest captures.

Table 1.—Grass-feeding Lepidoptera from light traps.

FAMILY	SCIENTIFIC NAME	FOOTHILL TRAP	VALLEY TRAP	TOTAL	EMERGENCE TRAP RECORDS	LITERATURE INDICATING GRASSES AS HOSTS
Arctiidae	*Estigmene acrea* (Drury)[a]	3	8	11		Borror & Delong (1955)
Crambidae	*Crambus topiarius* Zell.[a]	598	76	674	Merion bluegrass Delta bluegrass Pennlawn Creeping red fescue	Felt (1894); Fernald (1896); Forbes (1923); Johansen & Brannon (1955)
	C. plumbifimbriellus Dyar[a]	32	218	250	Astoria bentgrass Pencross bentgrass Delta bluegrass	Johansen & Brannon (1955)
	C. dorsipunctellus Kft.[a]	198	12	210	Pencross bentgrass	
	C. trisectus (Wlk.)[a]	16	120	136		Ainslie (1927); Fernald (1896); Forbes (1923); Miller (1940)
	C. leachellus cypridalis Hlst.[a]	20	46	66		Felt (1894); Forbes (1923)
	C. vulgivagellus Clem.[a]	5	0	5		Felt (1894); Forbes (1923); Miller (1940)
	C. pascuellus (L.)[a]	1	0	1	Astoria bentgrass	Fernald (1896); Forbes (1923)
	C. bonifatellus (Hlst.)[a]	0	1	1		Bohart (1947)
Gelechiidae	*Chionodes psiloptera* (Busck)	1,268	5	1,273	Merion bluegrass	
Noctuidae	*Feltia ducens* (Wlk.)	564	1,356	1,920	Astoria bentgrass Pennlawn	Crosby & Leonard (1918); Crumb (1929, 1956)
	Protagrotis obscura B & McD.	1,368	460	1,828	Delta bluegrass	
	Caenurgina erechtea (Cram.)[a]	118	115	233		Crumb (1956)
	Platyperigea extima (Wlk.)[b]	112	79	191	Merion bluegrass Pennlawn	Crumb (1956)
	Crymodes devastator (Brace)	37	52	89	Merion bluegrass Delta bluegrass	Crosby & Leonard (1918); Crumb (1929, 1956)
	Leucania[c] *insueta heterodoxa* Sm.	44	6	50		
	L. farcta (Grt.)	9	3	12		
	L. multilinea Wlk.	2	9	11		
	L. luteopallens Sm.	7	4	11		
	Graphiphora c-nigrum (L.)	13	32	45	Delta bluegrass Pennlawn	Crosby & Leonard (1918); Crumb (1956)
	Nephelodes emmedonia (Cram.)	14	31	45	Astoria bentgrass Pennlawn	Crosby & Leonard (1918); Crumb (1956); Knutson (1944)
	Agrotis venerabilis (Wlk.)	7	30	37		Crumb (1956)
	Euxoa messoria (Harr.)	21	7	28	Merion bluegrass Astoria bentgrass	Crosby & Leonard (1918)
	E. ochrogaster Gn.	6	1	7	Intermediate wheatgrass	
	E. septentrionalis Wlk.	4	2	6	Intermediate wheatgrass	
	"Rhynchagrotis" exsertistigma (Morr.)[a]	13	9	22		Crumb (1956)
	"Rhynchagrotis" insularis Grt.[a]	0	1	1		Crumb (1956)
	Septis finitima (Gn.)	19	3	22		Crumb (1956)
	S. arctica	2	3	5		Crosby & Leonard (1918)
	S. cuculliformis (Grt.)[a]	3	1	4		Crumb (1956)
	Lacinipolia pensilis (Grt.)[b]	7	0	7	Delta bluegrass	Crumb (1956)
	Dargida procincta (Grt.)	4	3	7	Pennlawn	Crumb (1956)
	Agroperina dubitans (Wlk.)[a]	0	2	2	Delta bluegrass	Crumb (1956)
	Xylena nupera (Lint.)	1	1	2		Crumb (1956)
	Protoleucania albilinea (Hbn.)	1	0	1		Crumb (1956); Knutson (1944)
	Zosteropoda hirtipes	1	0	1		Crumb (1956)

[a] Authors list only grasses as hosts.
[b] Authors list food plants, but these do not include grasses. All other moths in this table have grasses listed as one food source, or have been shown by emergence-cage records to be associated with grasses.
[c] All *Leucania* species mentioned by Crumb (1956), including *L. farcta*, are at least grass feeders in part.

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Distribution Patterns of Young and Experienced Honey Bees Foraging on Alfalfa[1]

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ABSTRACT

Experiments were conducted in northern Utah to compare the dispersion patterns of young foragers and experienced field honey bees (*Apis mellifera* L.) from unaltered colonies when moved into a new area where alfalfa bloom was widespread. The experimental colonies were mass marked by introducing queens from a recessive mutant strain in which parts of the body normally black were cordovan brown. Few marked bees from newly transferred colonies were recovered more than 350 yards from their hives during the 1-week observation period. This fact applied to both the young and the experienced foragers. The number of marked bees recovered at one sampling station 1¼ miles from the apiary was comparable to the number collected adjacent to the apiary.

Many studies on the working habits of individual honey bees (*Apis mellifera* L.) have been reported. Sardar Singh (1950), Minderhoud (1931, 1951), Butler *et al.* (1943), Ribbands (1949), Weaver (1957), and others have shown that a bee may fly a considerable distance to collect pollen or nectar, but once there, she tends to confine herself to a small area, especially if the chosen species of flower is a good source of food. Research carried on in England by Ribbands (1951) and in Wyoming by Sturtevant & Farrar (1935) indicates that the distance between the apiary and the crop source affects the honey crop stored by the colonies. Eckert (1933), however, reported that the distance could increase to 2 miles before any detrimental effect on the honey yield could be observed.

Although these reports are of considerable interest and importance, they provide little information on the behavior of the entire field force of a colony or apiary. However, Butler *et al.* (*loc. cit.*) did observe that the nearer dishes received more visitors when sugar syrup was offered at distances from 160 to 400 yards.

STUDIES OF THE FORAGING RANGE.—Peer (1955), studying the foraging range of honey bees in Wisconsin, reported that the bees under observation foraged up to 3½ miles, but most of them were found in all directions within 2½ miles of their colonies. He estimated that 75% of the foragers were working beyond 1 mile from the apiary.

The directional orientation described by Eckert (1933) was observed by Linsley & MacSwain (1947) in California. They found that honey bees were not working in the field in which their hives were placed but frequently had become oriented in a particular direction to nearby fields.

In 1956 experiments were initiated to study under various conditions the distribution patterns of honey bees foraging on alfalfa in northern Utah. The main objective of the first experiment herein reported was to compare the dispersion pattern of young foragers undertaking field duties for the first time with that of the normal field force of bees of all ages after having been moved into a new area.

A genetically marked strain called cordovan was used for the experimental bees as they can be readily differentiated from the black, yellow, or hybrid strains common to the experimental area. The colonies used were requeened 10 to 12 weeks before the experiment with cordovan queens from the U. S. Department of Agriculture Bee Culture Station at Baton Rouge, Louisiana. By the time they were needed, practically all the bees in the colonies were of this strain.

The two phases of the test were conducted at two locations about 10 miles west of Logan. Several hundred acres of second-crop seed alfalfa in various stages of bloom and about 150 colonies were within a 2-mile radius of the marked colonies.

For one phase 10 cordovan colonies with unaltered populations were moved to the north edge of a large planting of alfalfa. More or less continuous bloom was available for a mile to the south of the bees, and to the north, east, and west were fields of grain or fallowed ground. Several other plantings farther south were sampled (fig. 1). Bees were also collected on a 30-acre field of alfalfa 1¼ miles to the northwest of the apiary. This field was the closest source of nectar to the bees in that direction. Samples were collected at various distances twice a day for the first 2 days and once a day for the rest of the sampling period.

In preparation for the phase with inexperienced foragers, 10 cordovan colonies located in North Ogden, 50 miles from the test area, were deprived of their normal field forces by moving the colonies on 2 successive days

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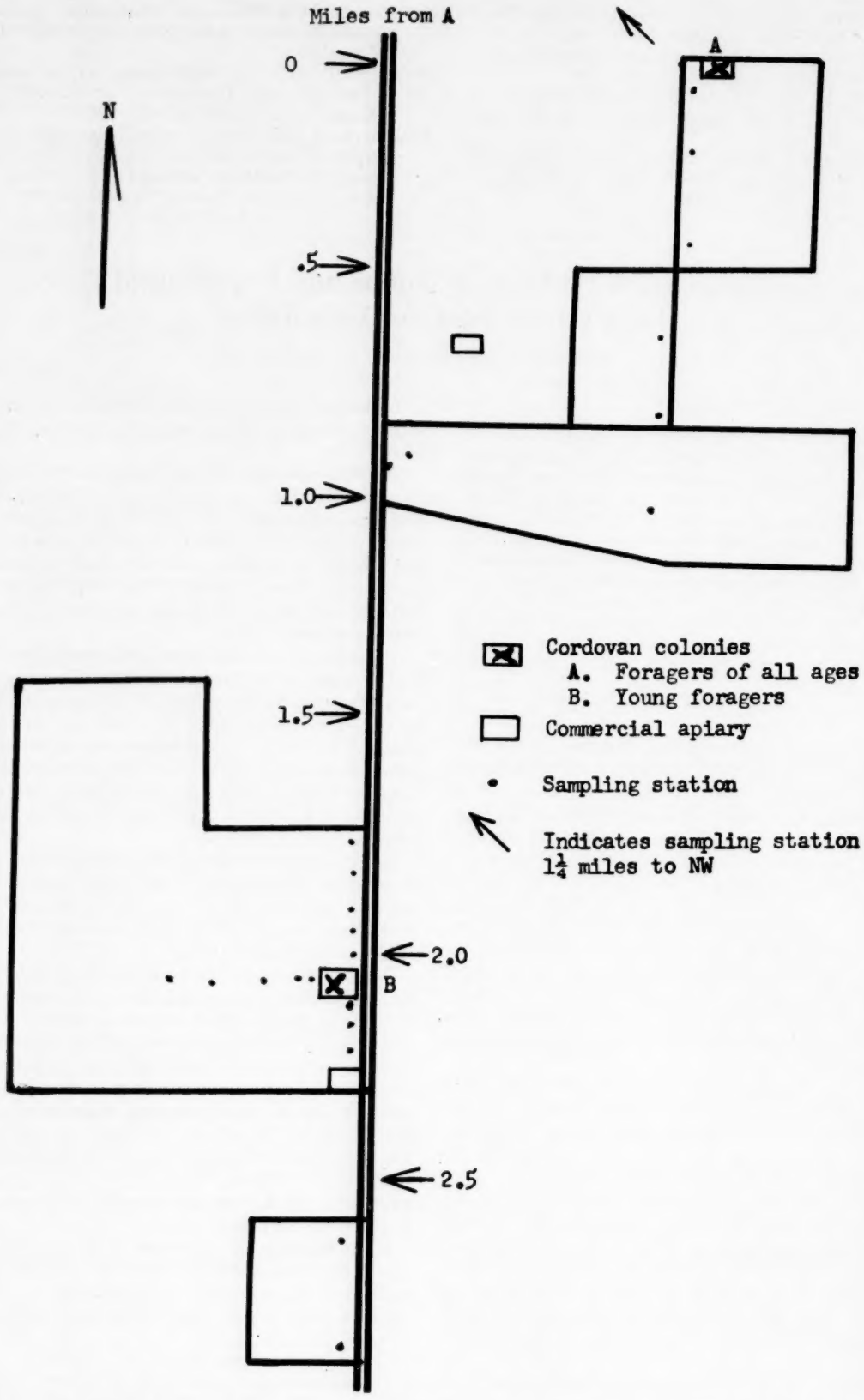

FIG. 1.—Diagram of experimental fields and location of test colonies, Petersboro, Utah. 1956. (Not to scale.)

during the peak of flight activity. Each time they were moved a few hundred yards away and the field bees were collected at their previous colony sites. Thus, the colonies were depleted of most of the bees of foraging age. On the third day the colonies were moved to the test area.

About 2 miles from the site of the first phase of the experiment, the 10 colonies deprived of their foragers were placed at the eastern edge of a 150-acre field of second-crop alfalfa. One portion of it was cut for hay during the sampling period because of the high plant bug (*Lygus* spp.) and armyworm (*Pseudaletia unipuncta* (Haw.)) populations. As in the previous phase, samples of bees were collected in the morning and afternoon during the first 2 days and at 24-hour intervals at midday thereafter. Three directions (north, south, and west) up to 450 yards were sampled. The limits of the field prevented further sampling. The data for all three directions were averaged for each distance. Samples at each distance beyond 450 yards represent only one direction.

At all sampling stations 25 to 150 field bees were collected with an insect net each day. The bees were killed and examined after all collections were made to establish the percentage of cordovan bees at each station. The area covered at each station was approximately 25 to 50 square yards. The fluctuation in number of bees collected was due in part to variations in populations.

DISCUSSION OF RESULTS.—At the first location most of the bees from the unaltered colonies were recovered within 350 yards of the apiary (table 1). Their greatest dispersal was reached 1½ days after being moved. Only a few cordovans were recovered beyond 700 yards, except on the field 1¼ miles to the northwest. Here they made up a substantial porportion of the bees on the field, especially toward the end of the sampling period.

Young bees undertaking foraging duties for the first time dispersed similarly to more experienced foragers from undisturbed colonies (table 2). Most of the young bees were recovered within 300 yards. At the end of the second day most of them were still working close to the hive, but some were found as far as 450 yards. Sampling was continued until 8 days after the move, but only an occasional cordovan bee was recovered more than ½ mile from the apiary.

Table 1.—Percentage of cordovan bees of all ages collected at progressive distances from the test apiary; colonies moved on the morning of August 6, 1956.

DISTANCE	AUGUST 6		AUGUST 7		AUGUST 8	AUGUST 9	AUGUST 10
	A.M.	P.M.	A.M.	P.M.			
Yards							
100	27.6	45.0	42.4	12.5	17.7	14.0	6.1
350	25.4	22.5	21.3	11.7	7.8	4.9	8.1
700	1.6	2.4	2.8	1.6	2.5	1.1	—
1,056	—	1.3	0	0	1.8	4.8	—[a]
1,480	0	1.9	1.5	0	1.5	0	—[a]
1,760	0	0	1.4	0	2.0	0	0
Miles							
1¼ NW	6.5	8.1	1.3	8.9	8.2	16.8	14.7
1½ SW	0	1.2	0	0	0.61	0.8	0
2½ SW	0	0	0	0	0	0	0

[a] Field cut.

Table 2.—Percentage of young cordovan foragers collected at progressive distances from the test apiary; colonies moved on the morning of July 26, 1956.

DISTANCE	JULY 26		JULY 27		JULY 28	JULY 29	JULY 30
	A.M.	P.M.	A.M.	P.M.			
Yards[a]							
50	0.93	15.7	31.2	23.9	15.3	22.1	37.0
100	2.10	21.6	25.0	24.7	18.8	7.4	15.1
200	2.3	8.4	7.8	16.5	10.9	5.6	21.9
300	0	5.2	6.9	4.8	5.2	4.6	5.4
450	0	0	3.6	6.1	0	2.4	4.1[c]
Miles[b]							
¼ S	0	1.3	0	0	1.4	0.96	1.1
¾ S	0	0	0	0	0	0	0
1 N	0	0	0	0	0	0	0
1½ N	0	0	0	0	0	0	0.7

[a] Each distance represents an average of three directions until after July 27, when cutting of one field left only two directions.

[b] One sampling station at each distance.

[c] Collections made at this station on August 1 and 2 were composed of 8.8 and 1.2% cordovans, respectively.

In view of the expanding area encompassed as the distance from the marked colonies increased, it cannot be said that most of the foragers from the marked colonies were working within the 350-yard radius observed. However, the scarcity of marked bees at the more distant sampling stations may be more than just a matter of dilution over an expanding area. This possibility is strengthened by the fact that the marked bees visited alfalfa in bloom 1¼ miles away almost as freely as they foraged on alfalfa blooms adjacent to their hives when there was no other source of nectar closer in that direction.

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The Role of Granulated Insecticides for Control of Sweet Corn Pests in New Jersey[1]

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ABSTRACT

In order to determine the role of granulated insecticides in the sweet corn insect-control program in New Jersey, experiments were conducted during the 1954–57 seasons. Of the materials used, 2½% heptachlor on attaclay and 1% parathion on tobacco were the most efficient, giving excellent control of European corn borer (*Pyrausta nubilalis* (Hbn.)), corn earworm (*Heliothis zea* (Boddie)), and fall armyworm (*Laphygma frugiperda* (J. E. Smith)), on corn in the whorl stage. Sprays or dusts are still necessary when the plants are in the ear stage, but as a supplement the applications of granulated insecticides can be of great value to the New Jersey sweet corn growers.

The use of granulated insecticides was first reported by Wilson (1950), Vannote (1951) and Whitehead (1951) for control of mosquito larvae. Later, Farrar (1953) found that certain granulated formulations controlled corn earworm on field corn in the whorl stage, and since then, considerable experimental data, as reported by various workers, has shown that the European corn borer (*Pyrausta nubilalis* (Hbn.)) can be effectively controlled with several of these materials. Cox *et al.* (1956) tested numerous insecticides formulated with various types of granules and observed the effects of timing and rates of application. At the present time large acreages of corn, particularly in the mid-West, are treated with granulated formulations for corn-borer control.

In New Jersey, essentially the entire crop of sweet corn is sold to the fresh market. The high cost of production and the persistent consumer demand for clean ears necessitates a careful and efficient insect control program. At the present time, this goal is attained by means of high-clearance sprayers and dusters, with insect-control percentages usually running 95% or higher when proper application schedules are maintained. For the granulated formulations to enter the control program, their performance must be equal to or surpass the efficiency of the currently used materials.

Sweet corn is harvested in New Jersey from late June until frost. Planting starts in early April and extends through to mid-July. Thus corn in the whorl stages is present from May until September. It is on these stages of growth that the granulated materials are most efficient.

The six important foliage-feeding insect pests that the New Jersey sweet corn grower must contend with annually are flea beetles (*Chaetocnema* spp.), European corn borer, sap beetles (*Carpophilus* spp.), corn earworm (*Heliothis zea* (Boddie)) fall armyworm (*Laphygma frugiperda* (J. E. Smith)) and aphids (*Rhopalosiphum maidis* (Fitch)). Of these, the European corn borer, the corn earworm, and the fall armyworm, lend themselves to the action of applications of granulated insecticides. It has also been observed that aphids are controlled in the whorl when granulated phosphate materials are used. When the plants are in the tassel and ear stage, the granules are not sufficiently effective for any of these pests. Thus, the granules will not supplant the sprays or dusts, but they may be used effectively for whorl-stage applications. Many growers have fields of corn in various stages of growth and, in many cases, rather widely dispersed from the central farm. The use of a relatively inexpensive and easily transported applicator of granules would materially increase the ease and efficiency of the insect-control program.

Table 1.—Results of first-generation test on control of the European Corn Borer (ECB) in 1954.

Treatment	Dates of Application (June)	E.C.B./100 Plants		Per Cent Control
		Ear	Stalk	
1. Parathion (2%) on tobacco	12, 17, 22, 28	0	0	100
2.	12, 22	0	0	100
3. Parathion (2½%) on Celite[a]	12	—	—	—
4. Endrin (1%) on tobacco	12, 17, 22, 28	0	0	100
5.	12, 22	0	0	100
6. Dieldrin (2%) on tobacco	12, 17, 22, 28	4	0	97
7.	12, 22	0	0	100
8. DDT (5%) on Celite[a]	12	—	—	—
9. Endrin (1%) dust	12, 17, 22, 28	0	4	97
10. Untreated check		4	112	—

[a] Treatments 3 and 8 were phytotoxic, so the plots were discontinued.

Table 2.—Results of first-generation test on control of European Corn Borer (ECB) in 1955.

Treatment	Dates of Application (June)	E.C.B./100 Plants		Per Cent Control
		Ear	Stalk	
1. Parathion (2%) on tobacco	8, 13, 17, 22	0	0	100
2.	8, 17	4	2	98
3.	8, 22	0	0	100
4. Endrin (1%) on tobacco[a]	8	—	—	—
5. DDT (5%) on attaclay (impregnated)	8, 13, 17, 22	10	16	91
6.	8, 17	16	56	77
7.	8, 22	2	48	84
8. DDT (5%) on attaclay (fused)[a]	8	—	—	—
9. DDT (5%) dust	8, 13, 17, 22	0	0	100
10. Parathion (1%) dust	17, 22	2	0	99
11. Endrin (1%) dust[a]	8	—	—	—
12. Untreated check		108	198	—

[a] Treatments 4, 8, and 11 were phytotoxic, so the plots were discontinued.

In order to determine the role of granulated insecticides in the New Jersey sweet-corn program, considerable work, primarily for European-corn-borer control, has been conducted. Insect control, timing of applications, and phytotoxicity were the chief factors observed. The tests were initiated in 1954 and continued through 1957. The tests made each season are outlined below.

1954.—The materials listed in table 1 were applied to the Marcross 13-6 hybrid sweet corn grown on plots five rows wide by 30 feet long replicated three times. Application rates of 25 to 30 pounds per acre were made using a knapsack-type puff duster. A 5-day and a 10-day schedule were maintained, and at harvest 50 plants from each treatment were examined.

1955.—The materials listed in table 2 were applied to Carmel Cross hybrid sweet corn grown in plots four rows wide by 50 feet long replicated four times in a modified latin-square arrangement, with eight check plots in the

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Table 3.—Results of first generation test on control of European Corn Borer (ECB) in 1956.

Treatment	Dates of Application (June)	E.C.B./100 Plants		Per Cent	
		Ear	Stalk	Control	Clean Ears
1. Parathion (1%) on tobacco	11, 21	76	94	90.3	54
2.	11, 26	60	114	90.1	56
3. Parathion (2%) on tobacco	11, 21	50	128	89.8	62
4.	11, 26	88	122	88.0	40
5. Parathion (1%) on attaclay[a]	11	—	—	—	—
6. Parathion (2%) on attaclay[a]	11	—	—	—	—
7. DDT (5%) fused on tobacco	11, 21	62	164	87.1	56
8.	11, 26	78	266	80.5	42
9. DDT (5%) solvent impregnated on tobacco	11, 21	46	152	88.7	58
10.	11, 26	54	160	87.8	60
11. Malathion (4%) on tobacco	11, 21	376	302	61.3	36
12.	11, 26	100	338	75.0	36
13. Malathion 5% on Pike's Peak clay[a]	11	—	—	—	—
14. Endrin (1%) on attaclay[a]	11	—	—	—	—
15. Heptachlor (2%) on tobacco	11, 21	64	132	88.8	52
16.	11, 26	128	306	75.2	22
17. Heptachlor (2½%) on attaclay	11, 21	26	112	92.1	74
18.	11, 26	24	88	93.6	76
19. Untreated check		474	1,276	—	0

[a] Treatments 5, 6, 13, 14 were phytotoxic, so plots were discontinued.

field design. A 5-day, a 10-day, and a 15-day schedule were used. The granulated materials were applied at 20 to 25 pounds per acre and the dusts at 40 pounds per acre using a knapsack puff duster. Fifty plants from each treatment were examined. All the granulated formulations were 30 to 60 mesh.

The first application was made when 32 egg masses per 100 plants were found.

1956.—The materials listed in table 3 were applied to Carmel Cross hybrid sweet corn using a knapsack puff duster. The plots were four rows wide by 40 feet long, replicated four times in a modified latin-square arrangement, with eight check plots throughout the field design. A 10-day and a 15-day schedule were used with 25 to 30 pounds of granules applied at each application. Fifty plants per treatment were examined at harvest for insect injury. All the formulations were on 30 to 60-mesh granules.

The first application was made when 87 egg masses per 100 plants were present with about 2% of them emerged.

1957.—The materials listed in table 4 were applied to Carmel Cross hybrid sweet corn grown in plots four rows wide by 82 feet long, replicated three times using a knapsack-type puff duster. One application was made at the rate of 25 to 30 pounds per acre. Owing to severe drought conditions, growth of the corn was poor, consequently no ear records were taken. A count of the number of plants in each plot was made and a record of those injured by European corn borer was obtained.

In this test the application was delayed until most of the egg masses had hatched and the larvae had moved into the whorls of the plants.

Table 4.—Results of first-generation test for control of the European Corn Borer in 1957.

Treatment	Total No. Plants	No. Plants Injured	Per Cent Injury
1. Parathion (1%) on tobacco	1,428	23	1.5
2. Heptachlor (2½%) on attaclay	1,417	24	1.7
3. Endrin (1%) on attaclay[a]	1,463	22	1.5
4. Heptachlor (2%) on tobacco	1,435	54	3.7
5. DDT (5%) on tobacco	1,549	165	10.6
6. DDT (5%) on attaclay	1,369	145	10.6
7. DDT (5%) on bentonite[a]	1,417	110	7.7
8. Untreated check	1,282	714	55.8

[a] Treatment 3 caused severe injury to plants and 7 showed some phytotoxicity.

CORN EARWORM AND FALL ARMYWORM RESEARCH.—The occurrence of corn earworm and fall armyworm on young corn in New Jersey, especially during August and September, often poses a serious problem and, without adequate control measures, yields can be reduced to a considerable degree.

DDT sprays and dusts, using 1 to 2 lbs. of actual toxicant per acre applied to the pretassel stage, have been fairly successful, depending on the degree of infestation and the thoroughness of the applications. Often two or three treatments are required for adequate control. One of the complicating factors involved is that the grower is often not aware of the presence of these pests until they have buried themselves deep into the whorls and have seriously damaged the young developing tassels.

On several occasions during the 1954–58 seasons, the author had the opportunity to test several of the granulated formulations. Hand applications with a knapsack puff duster and machine treatments with a Gandy Hi-Lo applicator were made. Of the materials tested, parathion at 1 or 2% on tobacco and heptachlor at 2½% on attaclay applied at the rate of 25 to 30 pounds per acre were the most effective. In every test, one application gave excellent control for a period of 2 to 3 weeks. If reinfestation occurred before the tassels had emerged, a second treatment was made. After tassel emergence, better control was obtained with a spray application using a DDT-parathion (2 qts. 25%, ½ pt. 42%) or DDT-malathion (2 qts. 25%, 1 pt. 57%) formulation per acre.

With aphids present, the parathion granules were superior, since they curtailed buildup in the whorls and prevented tassel damage until the sprays were applied. It was also noted, both in the corn borer- and earworm- fall armyworm-tests that the incidence of sap beetles was greatly curtailed where the granules were used.

DISCUSSION OF RESULTS.—The results of the various tests with granulated insecticides for sweet-corn insects, especially the European corn borer, were as follows:

1954.—A 10-day schedule using parathion, endrin, and dieldrin on tobacco granules was as effective as a 1% endrin dust used at 5-day intervals. Both parathion and DDT on Celite granules were phytotoxic.

1955.—Parathion on tobacco granules used on a 15-day schedule was as effective as a DDT or a parathion dust applied at 5-day intervals.

DDT at 5% impregnated on attaclay granules was not as effective on a 5-, 10-, or 15-day schedule as were the parathion granules or dusts.

Endrin on tobacco, DDT fused on attaclay granules, and endrin dust were phytotoxic.

1956.—This year marked the heaviest infestation encountered during the 4-year test, with a total of 1,750 borers per 100 plants occurring on the untreated checks. The 2½% heptachlor on attaclay was the most efficient deterrent, with the 15-day treatments being as effective as the 10-day. The parathion treatments on tobacco formulations were slightly less effective. From the standpoint

of clean ears, however, the heptachlor treatment was considerably better than the parathion. The 2% heptachlor on tobacco granules was not so efficient as the attaclay formulation, especially with respect to clean ears. The DDT formulations were about as good as the parathion treatments, although the fused DDT material on the 15-day treatment was not so effective as the solvent-impregnated DDT both, from the standpoint of control and per cent of clean ears.

The parathion on attaclay, malathion on Pike's Peak clay, and endrin on attaclay were all phytotoxic. It is interesting to note that the malathion on tobacco showed no phytotoxicity.

The first application was made when only 2% of the egg masses had hatched. Subsequent experience has shown that the timing was incorrect. A larger percentage of the larvae should have emerged before the first treatment was made.

1957.—The parathion on tobacco and heptachlor on attaclay again were the most efficient treatments, with the DDT formulations somewhat less effective. The heptachlor on tobacco was again not quite so effective as the heptachlor-attaclay formulation. Endrin on attaclay and DDT on bentonite were phytotoxic.

The results of this work show that several granulated insecticides are effective for the control of certain insect pests on sweet corn in New Jersey. Of the materials tested, heptachlor on attaclay and parathion on tobacco were the most efficient.

Although the endrin formulations used gave excellent control of the European corn borer, the plant damage that they caused in most of the tests precludes its being recommended, at least under New Jersey conditions. Certain formulations of DDT and parathion were also phytotoxic while others were safe in this respect. The phytotoxicity encountered with some of the formulations is not necessarily due to the insecticide itself. Other constituents of a particular formulation such as the solvent or the type of granule used could have been a contributing factor. It should also be considered that the variety of corn used in the experiment may have played a role.

Timing of application is important. When the treatment is delayed until most of the eggs have hatched, a single application of a suitable granulated formulation will give excellent control under the corn-borer conditions usually encountered in New Jersey. When infestations are severe, two applications 10 to 15 days apart will suffice.

Experience has shown that one or two applications of granules are as effective as three to five applications of a conventional dust or spray. Although timing of application with the granules is important, it is not so critical as are the sprays or dusts, where the material must be applied before the young borers enter the plant.

When the corn is in the whorl stage, excellent control of corn earworm, fall armyworm, and aphids can be obtained with suitable granulated formulations. Under the sweet-corn-growing program existing in New Jersey, these pests are often a serious factor.

However, the granulated insecticides will not give adequate control throughout the season. Sprays or dusts are necessary when the corn plants are in the ear stage. As a supplement to the spray or dust program, the granulated materials can be of great value to the grower in several ways: (1) on farms where multiple plantings occur, granules can be used on the whorl-stage corn, thus releasing the big high-clearance machines for the critical job of protecting the maturing fields from earworm attack; (2) the applicators of granules are inexpensive and easier to move from field to field, especially if the fields are separated by some distance; (3) with timing of application not so critical as with sprays or dusts, a greater acreage can be treated with much less effort and with less danger of being too late; (4) although proper adjustment and calibration of the granulated insecticide machines are necessary, the actual operation is not so critical as that of the more complex high-clearance spray and dust equipment, thus less skillful operators can be used satisfactorily; and (5) although the granulated formulations are more expensive per acre application, the fewer treatments that are ordinarily required, in contrast to the dusts or sprays, actually make them less costly.

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Field Experiments for Control of Late-Season Infestations of Several Cotton Insects[1]

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Abstract

Sevin® (1-naphthyl-*N*-methyl carbamate) applied at 1.0 and 1.6 pounds per acre in dusts and at 1.5 pound per acre in a spray gave effective control of the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)) in field-plot experiments conducted in 1958. Sprays containing calcium arsenate at 7 pounds, Monsanto CP-7769 (hexaethyl (ethylthiomethylidine) triphosphonate) at 0.5 pound, or dicapthon at 1.0 pound per acre were as effective in boll weevil control as dieldrin at 0.38 pound or Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) at 0.25 pound per acre. Calcium arsenate plus DDT gave poor control of the bollworm. Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), ethion, and Delnav® (2,3-*p*-dioxanedithio' *S,S*-bis(*O,O*-diethyl phosphorodithioate) at 0.5 pound, demeton and CP-7769 at 0.25 pound and Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) and dicapthon at 1.0 pound per acre were equally effective against the desert spider mite (*Tetranychus desertorum* (Banks)) for 7 days after treatment. Sprays containing Sevin at 1 pound, toxaphene at 2 pounds plus DDT at 1 pound, malathion or dicapthon at 0.38 pound, or endrin at 0.3 pound per acre were effective against the cotton leafworm (*Alabama argillacea* (Hbn.)). Sevin dust at 0.9 pound gave only fair control and that obtained with CP-7769 spray at 0.25 pound per acre was poor.

Heavy late-season infestations of several cotton insects in central Texas in 1958 afforded an opportunity to conduct field experiments with some of the new insecticides that showed promise against them in 1957 (Cowan *et al.* 1958). The predominating species were the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)). The desert spider mite (*Tetranychus desertorum* (Banks)) was of less importance. Infestations of the cotton leaf worm (*Alabama argillacea* (Hbn.)) also became sufficiently heavy late in 1957 and 1958 for similar tests to be made.

General Procedure.—In the experiments with the boll weevil, bollworm, and desert spider mite the plots were 16 rows wide and of sufficient length to make 0.2 acre. They were arranged in randomized blocks with four replications of treatments except in experiment 4, in which there were only three. In the spider mite experiment untreated plots were not included within the experimental design, but were located in the same field adjacent to the treated plots. In the first cotton leafworm experiment the plots were eight rows wide and of sufficient length to cover 0.1 acre, arranged in randomized blocks with three replications of treatments, and in the second experiment the plots were twice as large, with no replications.

Methods of applying insecticides were the same as those previously reported (Cowan *et al.* 1956). In the boll weevil-bollworm experiments applications were made at 5-day intervals except in experiment 4, in which the intervals were a few days longer following two irrigations. Only one application was made in the spider mite and cotton leafworm experiments.

The procedures for recording data were the same as those reported by Ewing & Parencia (1943), Cowan *et al.* (1958), and Parencia *et al.* (1954).

Control of Boll Weevils and Bollworms (Table 1).—In experiment 1 each insecticide reduced the boll weevil infestation significantly below that of the check. The poorest control was obtained with Sevin® (1-napthyl-*N*-methyl carbamate) spray at 0.9 pound per acre. In bollworm control when boll injury was considered, Sevin dusts and the higher spray dosage were better than Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl phosphorodithioate) plus DDT. Spider mite infestation was greater following use of Sevin sprays than following Sevin-sulfur dusts or Guthion plus DDT spray. Each insecticide gave a significant increase in yield over the untreated check, and the greatest increase was from the higher dosage of Sevin dust.

In experiment 2 various phosphorus insecticides were added to the toxaphene-DDT mixture to determine whether they would increase its effectiveness against the boll weevil. Unfortunately dry weather reduced the fruiting period of cotton plants, making such an evaluation impossible. No significant difference in seasonal boll weevil control was evident between the various insecticide mixtures, and each of them reduced bollworm injury significantly below the check. Spider mite infestations were slightly higher following treatment with malathion plus DDT than with the other mixtures, but the difference was not significant. Each mixture gave a significant gain in yield over the untreated check, and toxaphene plus DDT was better than malathion plus DDT.

In experiment 3 each insecticide reduced the boll weevil infestation significantly below that of the untreated check. All the treatments except calcium arsenate-DDT spray gave significant reductions in bollworm injury, and dieldrin-DDT and dicapthon-DDT gave significantly better control than calcium arsenate-DDT. Each insecticide gave a significant gain in yield over the untreated check, and dieldrin-DDT was better than calcium arsenate-DDT. Owing to difficulty encountered in applying the calcium arsenate-DDT mixture, the total volume of spray was 15 gallons per acre whereas with the other insecticides it was 7.5 gallons.

Experiment 4 was conducted in a field that received two irrigations. When the experiment was begun on July 3, the cotton fleahopper (*Psallus seriatus* (Reut.)) had already caused considerable damage. The boll weevil infestation was fairly light until August 5, but the bollworm infestation was so severe that the untreated checks

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[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Effectiveness of several insecticides applied as dusts and sprays against the boll weevil and bollworm in field-plot experiments.

Insecticide[a]	Active Ingredient (Lb./A.)	No. Spider Mites per Square Inch	Weevil Punctured Squares (%)	Bollworm-Injured		Seed Cotton (Lb./A.)	
				Squares (%)	Bolls (%)	Total	Gain Over Check
Experiment 1 (5 applications between June 27 and July 16)							
Sprays							
Sevin 2.5	0.9	0.64	30.4	2.9	3.3	918	258
	1.6	.77	18.6	1.4	2.2	1,007	347
Guthion 1.5+DDT 2	0.25+1.0	.10	17.5	2.8	5.0	927	267
Dusts							
Sevin 10+sulfur 40	1.0 +4.0	.26	15.3	0.9	1.4	1,040	380
	1.6 +6.4	.08	12.0	.8	1.8	1,191	531
Untreated	—	.41	59.6	2.4	4.2	660	—
L.S.D. at 5% level	—	.31	10.4	1.4	2.1	133	—
Experiment 2 (8 spray applications between July 1 and August 6)							
Toxaphene 4+DDT 2	2.0 +1.0	0.87	29.7	1.5	3.6	817	338
Toxaphene 4+DDT 2 +malathion 5	2.0 +1.0 +0.31	.86	27.1	1.4	3.5	734	255
Toxaphene 4+DDT 2 +methyl parathion 2	2.0 +1.0 +0.12	.70	27.1	2.7	3.7	718	239
Toxaphene 4+DDT 2 +Guthion 1.5	2.0 +1.0 +0.06	.54	24.6	1.3	3.2	726	247
Malathion 5+DDT 2	1.25+1.0	1.12	28.6	2.0	3.8	689	210
Untreated	—	0.89	58.4	3.5	7.9	479	—
L.S.D. at 5% level	—	—	8.4	1.1	1.7	89	—
Experiment 3 (7 spray applications between July 1 and August 5)							
CP-7769, 2+DDT 2	0.5 +0.9	0.004	28.8	3.9	5.6	774	206
Dieldrin 1.5+DDT 2	.38+ .9	.21	26.6	3.0	4.3	859	291
Dicapthon 2+DDT 2	1.0 + .9	.09	27.4	2.1	4.2	838	270
Calcium arsenate 7 +DDT 2	7.0 + .9	.62	26.5	5.8	6.5	742	174
Guthion 1.5+DDT 2	0.25+ .9	.03	23.8	3.2	5.3	790	222
Untreated	—	.41	45.6	4.4	8.0	568	—
L.S.D. at 5% level	—	.26	8.1	1.6	1.8	111	—
Experiment 4 (11 spray applications between July 3 and August 20)							
Toxaphene 4+DDT 2	2.0 +1.0	0.71	34.5	3.3	2.7	2,737	787
Endrin 1.6+DDT 2	0.35+0.35	.63	45.0	3.3	3.7	2,235	285
Heptachlor 1+DDT 2	.5 +1.0	.83	46.7	4.1	4.0	2,262	312
Heptachlor 1+DDT 2+methyl parathion 1	.25+0.25+0.9	.40	45.7	3.9	4.5	2,505	555
Methyl parathion 2 +DDT 2	.5 +1.0	.35	35.8	4.3	3.0	2,458	508
Untreated[b]	—	.92	62.5	6.1	7.1	1,950	—
L.S.D. at 5% level	—	—	17.2	1.1	1.6	—	—

[a] Pounds per gallon in sprays, per cent in dusts.
[b] Treated with DDT at 1.0 pound per acre 5 times between July 23 and August 12 for bollworm control.

had to be protected with DDT from July 23 to August 12 to prevent loss of all fruit, which would eliminate the boll weevil infestation. Weevil infestations became heavy on August 11. The field topography from north to south showed considerable slope; consequently, fallen infested squares were carried down the rows by irrigation water, resulting in a considerably higher weevil infestation in the plots located at the south end of the field. Each of the treatments except heptachlor plus DDT, and heptachlor plus DDT plus methyl parathion reduced the weevil infestation significantly below the untreated check, but there were no differences between treatments. Good bollworm control was obtained with each treatment.

Spider mite infestations were slightly lower in the plots treated with phosphorus compounds. The species *Tetranychus cinnabarinus* (Boisd.) was identified from cotton for the first time from McLennan County. All the other treatments gave significantly better control of a light infestation of the cabbage looper (*Trichoplusia ni* (Hbn.)) than heptachlor plus DDT.

After August 20 further treatment with high-clearance ground equipment could not be made without causing considerable mechanical injury to plants. Since continued treatment was needed to protect late bolls, 2 pounds of toxaphene plus 1 pound of DDT plus 0.25 pound of methyl parathion per acre were applied by airplane to all plots when the remainder of the field was treated by the grower on August 27 and September 1. The wide variation in boll weevil infestation between replicates caused considerable variation in yield, but differences between treatments were not significant.

Control of Spider Mites (Table 2).—There were no significant differences between demeton, ethion, Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Monsanto CP-7769 (hexaethyl (ethylthiomethylidyne) triphosphonate), Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol), Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), and dicapthon in the control of the desert spider mite 1, 4, and 7 days after treatment. After 11 days all the other

Table 2.—Effectiveness of several acaricides against the desert spider mite.

Acaricide[a] (Lb./Gal.)	Active Ingredient (Lb./A.)	Number of Spider Mites per Leaf: Before Treatment	After Treatment (Days) 1	4	7	11	Average
Demeton 2	0.25	71.7	1.2	0.01	0	0.02	0.3
Trithion 4	.5	69.6	0.2	.2	0	.03	.1
Ethion 4	.5	69.7	1.1	.3	0.04	.01	.4
Delnav 4	.5	83.2	3.0	.4	.03	.03	.8
CP-7769, 2	.25	72.1	1.1	.3	.05	.02	.4
Kelthane 1.6	1.0	69.1	4.9	1.0	1.7	1.0	2.2
Dicapthon 2	1.0	71.3	2.5	0.4	1.6	21.5	6.5
L.S.D. at 5% level	—	—	—	—	—	10.9	4.0
Untreated (outside check)	—	72.9	73.6	67.6	69.6	40.6	62.9

[a] One spray application on August 7.

acaricides gave better residual control than dicapthon.

CONTROL OF COTTON LEAFWORM (TABLE 3).—In experiment 1 conducted in 1957, both dosages of Sevin, CP-7769, and toxaphene-DDT applied as sprays reduced the infestation significantly below the check, but Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) did not.

In experiment 2 conducted in 1958 good control was obtained 48 hours after treatment with both Sevin formulations, toxaphene plus DDT, endrin, malathion, and dicapthon applied as sprays. Only fair control was obtained with Sevin dust. However, light showers on the day it was applied may have reduced its effectiveness. CP-7769 spray gave considerably poorer control in 1958 than in 1957.

Table 3.—Effectiveness of several insecticides against the cotton leafworm in field experiments.

Insecticide[a]	Active Ingredient (Lb./A.)	Number of Larvae per Plant: Before Treatment	After Treatment (Days) 1	2 or 4[b]	Average
Experiment 1 (1 spray application on August 1, 1957)					
Sevin 1	0.5	8.7	1.2	1.3	1.2
	1.0	8.2	0.3	0.6	0.4
Thiodan 4	0.5	10.3	3.6	5.3	4.4
CP-7769, 2	.25	9.9	2.9	1.7	2.3
Toxaphene 4+DDT 2	1.5+0.75	8.3	2.0	2.9	2.4
Untreated	—	10.6	6.0	9.1	7.6
L.S.D. at 5% level	—	—	3.2	4.3	3.6
Experiment 2 (1 application on September 17, 1958)					
Dust					
Sevin 10	0.9	12.9	3.3	2.9	3.1
Sprays					
Malathion 5	.375	11.1	1.1	0.5	0.8
Dicapthon 2	.375	11.7	1.8	.5	1.2
CP-7769, 2	.25	12.7	8.7	5.7	7.2
Sevin 2.5	1.0	12.4	0.2	0	0.1
4	1.0	13.5	.4	0.1	.2
Toxaphene 4+DDT 2	2.0+1.0	15.2	1.3	.1	.7
Endrin 1.6	0.3	11.8	0.8	.2	.5
Untreated	—	14.1	13.2	13.2	13.2

[a] Pounds per gallon in sprays, per cent in dusts.
[b] 4 days in experiment 1, 2 days in experiment 2.

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Effect of Varying Conditions in a Laboratory Testing Technique on the Mortality of Mosquito Larvae[1]

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ABSTRACT

The mortality of mosquito larvae increased as the volume of acetone-water suspensions or solutions of dieldrin, lindane, malathion, and DDT was increased from 100 to 1,000 ml. The increase was greater and more consistent with DDT than with the other larvicides and with larvae of *Anopheles quadrimaculatus* Say and *Aedes taeniorhynchus* (Wied.) than with *Aedes aegypti* (L.). No increase in mortality was caused with parathion. When the diameter of the test containers was increased from 3 to 6 inches but the concentration and volume of the suspensions or solutions were constant, mortality of *quadrimaculatus* decreased when dieldrin, lindane, and DDT were used, but not with malathion or parathion. No difference in mortality was observed with the other two species of larvae.

The use of various procedures in assaying the susceptibility of mosquito larvae to new insecticides as well as in resistance surveys has focused attention on the differences that may be obtained when certain test conditions are changed. The results obtained with different techniques must be considered in designing standard testing procedures and in attempting to correlate data from different sources and techniques. Some research showing the effect of various factors on the mortality obtained in tests with mosquito larvae has been reported in the literature (Krusé *et al.* 1952, Shidrawi 1957, Jones 1957, Parker 1957, Schmidt & Weidhaas 1958, Weidhaas & Gahan 1958). Although these workers showed differences with changes in technique, none of their experiments were made with a variety of insecticides against a range of species.

The experiments reported in this paper were conducted with three species of mosquito larvae and five larvicides—two organophosphorus materials (parathion and malathion) and three chlorinated hydrocarbons (dieldrin, lindane, and DDT). The effects of varying the volume of the suspensions or solutions and the size of the container were studied. Since depth and/or surface

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area of the testing medium varied with the volume or container size, some information became available on these two factors.

EXPERIMENTAL PROCEDURES.—Fourth-instar larvae of *Anopheles quadrimaculatus* Say, *Aedes taeniorhynchus* (Wied.), and *Aedes aegypti* (L.), used in these tests, were obtained from laboratory colonies. The insecticides used were dieldrin, lindane, parathion, malathion, and radioactive DDT (*p,p'*-DDT-4-C^{14} having an activity of 2.1 μc./mg.). In each test 25 larvae were exposed in a suspension or solution of the insecticide (depending upon its solubility in water) made by adding a concentrated acetone solution to water. Mortality readings were taken after 24 hours. The amount of C^{14}-labeled DDT present in the larvae at the end of the exposure period was determined in some tests. The procedures for radiometric analysis have been described (Schmidt & Weidhaas 1958). All tests were run in triplicate.

EFFECT OF VARYING VOLUME OF SUSPENSION OR SOLUTION.—In the first series of tests the concentration of the toxicants was held constant as the volume of the suspensions was varied from 100 to 1,000 ml. Volumes ranging from 100 to 400 ml. were used in 3-inch diameter glass jars, 500-ml. volumes in 3¼-inch (600-ml.) beakers, and 1,000 ml. volumes in 4-inch (1,000-ml.) beakers. The depth of liquid in the containers increased with the volume; the surface area was constant up to 400 ml. but increased when 500 and 1,000 ml. were used.

The results of tests with four of the insecticides are shown in table 1. With parathion no significant change in mortality was noted for any species when the volume was increased tenfold. With malathion, lindane, and dieldrin there was a slight increase with all three species, at least at one concentration. The results with DDT are shown in table 2. The mortality of all three species increased with the volume of suspension. The difference from the smallest to the largest volume was greatest with *quadrimaculatus* and least with *aegypti*; *taeniorhynchus* showed slightly less difference than *quadrimaculatus*. The dose absorbed by the larvae generally increased with the volume. However, this increase was small and with *taeniorhynchus* it was erratic.

Table 1.—Mortality of larvae of three species of mosquitoes exposed to different volumes of insecticide suspensions or solutions.

Insecticide	Concentration (p.p.m.)	Per Cent Mortality at Indicated Volume (Ml.)						
		100	150	200	250	400	500	1,000
Anopheles quadrimaculatus								
Parathion	0.006	84	—	80	84	—	81	89
Malathion	0.075	72	—	89	89	—	96	97
Lindane	0.05	24	27	—	37	—	64	—
Dieldrin	0.01	2	6	—	12	26	—	—
	0.025	16	18	—	32	42	—	—
Aedes taeniorhynchus								
Parathion	0.002	19	—	21	17	—	20	17
	0.004	93	—	97	97	—	99	93
Malathion	0.025	56	—	61	65	—	70	73
Lindane	0.025	65	—	64	68	—	75	68
	0.05	88	—	92	88	—	91	100
Dieldrin	0.05	76	—	83	77	—	77	—
	0.08	84	—	89	95	—	100	100
Aedes aegypti								
Parathion	0.0075	83	—	95	77	—	80	81
	0.01	99	—	99	100	—	100	98
Malathion	0.04	28	—	12	21	—	29	25
	0.07	39	—	60	53	—	63	64
Lindane	0.04	68	—	77	70	—	85	89
Dieldrin	0.01	55	—	75	84	—	87	85

Table 2.—Mortality of larvae of three species of mosquitoes exposed to various volumes of C^{14}-DDT suspensions, and amount of DDT absorbed.

Volume of Suspension (Ml.)	*Anopheles quadrimaculatus* (0.007 p.p.m.)[a]		*Aedes taeniorhynchus* (0.075 p.p.m.)[a]		*Aedes aegypti* (0.0025 p.p.m.)[a]	
	Mortality (%)	Dose Absorbed (μg./larva)	Mortality (%)	Dose Absorbed (μg./larva)	Mortality (%)	Dose Absorbed (μg./larva)
100	22	0.005	12	0.056	63	0.002
200	63	.006	12	.068	77	.002
250	70	.007	28	.050	84	.002
500	96	.007	37	.078	83	.003
1,000	92	.008	72	.068	85	.003

[a] Concentration of DDT in suspension.

EFFECT OF VARYING THE CONTAINER SIZE.—In the second series glass containers of four different diameters were used—the regular 3-inch testing jars, 3¼-inch beakers, 4-inch beakers, and 6-inch battery jars—but the concentration of the toxicants, number of larvae, and volume of suspension were held constant. The results are shown in tables 3 and 4. When parathion and malathion were used, no changes in mortality were noted. With lindane and dieldrin no change was seen in *aegypti* or *taeniorhynchus*, but the mortality of *quadrimaculatus* showed a progressive decrease. With DDT the mortality of *quadrimaculatus* decreased from 92% in the 3-inch jar to 32% in the 6-inch battery jar. With *taeniorhynchus* no decrease appeared and with *aegypti* only the 6-inch jar showed a decrease. When the diameter increased from 3 to 6 inches, the dose absorbed decreased slightly with *quadrimaculatus* and *aegypti* and considerably with *taeniorhynchus*.

DISCUSSION.—This investigation showed that the effect of different test conditions on mortality varied with the larvicide and with the mosquito species.

With parathion no change in mortality with any species was observed with changes in volume or size of container. The concentration is known to influence the

Table 3.—Mortality of larvae of three species of mosquitoes exposed to 250 ml. of insecticide suspensions or solutions in containers of different sizes.

Insecticide	Concentration (p.p.m.)	Per Cent Mortality in Container of Indicated Diameter (In.)			
		3	3¼	4	6
Anopheles quadrimaculatus					
Parathion	0.006	60	60	—	52
Malathion	.15	92	92	—	92
Lindane	.075	52	40	—	8
Dieldrin	.075	64	32	—	8
Aedes taeniorhynchus					
Parathion	0.002	12	15	15	13
Malathion	.025	73	70	68	74
Lindane	.02	65	60	59	55
Dieldrin	.05	79	81	72	76
Aedes aegypti					
Parathion	0.006	20	8	15	17
	.01	96	95	96	95
Malathion	.05	31	28	33	35
Lindane	.04	44	48	37	35
Dieldrin	.008	48	60	65	39

Table 4.—Mortality of larvae of three species of mosquitoes exposed to 250 ml. of C^{14}-DDT suspensions in containers of various sizes and amount of DDT absorbed.

Diameter of Container (Inches)	*Anopheles quadrimaculatus* (0.007 p.p.m.)[a]		*Aedes taeniorhynchus* (0.075 p.p.m.)[a]		*Aedes aegypti* (0.0025 p.p.m.)[a]	
	Mortality (%)	Dose Absorbed (μg./larva)	Mortality (%)	Dose Absorbed (μg./larva)	Mortality (%)	Dose Absorbed (μg./larva)
3	92	0.005	23	0.077	65	0.005
3.25	90	.005	20	.076	65	.005
4	78	.004	32	.045	71	.005
6	32	.004	23	.040	40	.003

[a] Concentration of DDT in suspension.

mortality, but increasing the total parathion available per larva by other means than increasing the concentration had no effect. It appeared that the parathion was in solution in the test jars and was equally distributed throughout and that the larvae did not remove enough parathion to diminish the concentration. In an earlier study (Schmidt & Weidhaas 1958) with two radioactive organophosphorus compounds, dimethoate and Co-Ral® (*O*-(3-chloro-4-methylumbelliferone *O,O*-diethyl phosphorothioate), a very small percentage of the chemical was found in the larvae.

With DDT the mortality of at least one species changed with variations in all test conditions studied. The results obtained with *quadrimaculatus* in these tests and in the earlier study showed that the mortality could be correlated with the concentration, volume of suspension, or number of larvae when one of these factors was varied while the other two were constant. Furthermore, the mortality could be correlated with the ratio of volume of suspension to number of larvae and with the total available DDT per larva. However, the regression line for total available DDT vs. mortality was steeper when the concentration was varied than when either volume or number of larvae was the variant. Bowman *et al.* (1959) have shown that DDT in the test jars is unequally distributed and that at a concentration of 0.01 p.p.m. approximately 50% of the DDT is lost in 24 hours by a process of codistillation with water vapor. Additional experiments (Weidhaas & Schmidt, in manuscript) have shown that *quadrimaculatus* and *aegypti* larvae have different dosage-mortality responses to DDT. Furthermore, it can be seen from the amounts of DDT absorbed that larvae removed an appreciable proportion of the toxicant from test suspension.

All these factors influence the variation in mortality with test conditions, and the results can be explained on the basis of the following factors: (1) Distribution of DDT initially and over the time the larvae are exposed will vary with container size, volume of suspension, and rate of water evaporation; (2) since different species may have different dosage-mortality relationships, the distribution or volatilization of DDT will have different effects on the mortality of different species; (3) the amount of toxicant the larvae remove will lower the effective concentration of the test suspension. Further studies are in progress to determine the distribution and loss of DDT from different containers at various concentrations.

It is interesting to note that, although mortality caused by DDT can be increased by increasing the test volume, decreasing the number of larvae, or by using narrower containers, this is not true with parathion, and is probably the result of the physical state of DDT in colloidal suspension.

Results with malathion, dieldrin, and lindane were intermediate between those obtained with DDT and parathion. Some variation in mortality was found with changing test conditions. Studies on the distribution, the absorbed dose, and the dosage-mortality response of different species of larvae to these toxicants are needed to show which factors influence the results.

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Tests of Insecticides for the Control of Goat Lice in 1957 and 1958[1]

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ABSTRACT

To investigate reports on resistance of goat biting lice (*Bovicola caprae* (Gurlt) and *B. limbatus* (Gerv.)) to chlorinated hydrocarbon insecticides, goats from two ranches near Kerrville, Texas, were dipped into insecticides at dosages recommended by the Entomology Research Division, U. S. Department of Agriculture. Lice from one ranch were not killed by 0.25% toxaphene, which previously had been very effective. Field tests were conducted with sprays of Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)), ronnel, malathion, Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Sevin® (1-naphthyl *N*-methyl carbamate), and toxaphene for the control of biting lice and sucking lice (*Linognathus stenopsis* (Burm.)). Toxaphene and 0.1% Delnav failed to kill lice. Others were effective within 24 hours on goats with fleece shorter than 10 weeks' growth, and from 3 to 5 days on goats with longer fleece. Flocks sprayed with 0.5% ronnel, 0.1% Bayer 21/199, and 0.5% Sevin were free of lice at the next shearing (2 to 4 months).

Angora goats raised in the Edwards Plateau area of southwestern Texas are commonly infested by three species of lice—the goat biting louse (*Bovicola caprae* (Gurlt)), the Angora goat biting louse (*B. limbatus* (Gerv.)), and the goat sucking louse (*Linognathus stenopsis* (Burm.)). Heavy infestations lower the vitality of the goats and reduce the quality and quantity of mohair.

Early recommendations by Babcock (1936) for the control of goat lice consisted of arsenic, coal-tar creosote, nicotine, and sulfur dips. Babcock (1944) and Parish & Rude (1946) reported that dips containing 0.2% of DDT were effective on freshly sheared goats or goats in full fleece. More recently Smith & Richards (1955) have shown that a number of chlorinated hydrocarbon and organic-phosphorus insecticides are effective against goat lice.

In 1957 reports began reaching the Kerrville laboratory that ranchers were not obtaining satisfactory control with sprays or dips of toxaphene, one of the most widely used insecticides for goat louse control. Studies were therefore undertaken to determine whether the lice had become resistant to toxaphene and to evaluate the effectiveness of newer insecticides against lice from problem ranches. The results of tests conducted in 1957 and 1958 are presented herein.

PRELIMINARY TESTS.—Precise evaluation of an insecticide is seldom feasible under field conditions, especially where resistance is suspected. Therefore, 40 goats heavily infested with biting lice from each of two ranches where toxaphene treatments reportedly had failed were moved to the laboratory so that they could be treated, isolated in groups, and examined individually at prescribed intervals.

Four dips were tested on 10 goats from each ranch—two toxaphene formulations, DDT, and a mixture of toxaphene and lindane on those from ranch 1; and toxaphene, DDT, methoxychlor, and lindane on those from ranch 2.

Each goat was completely submerged in a 50-gallon portable vat. After treatment each group was isolated in a separate pen to avoid contamination between treatments. The goats were examined for lice before treatment and several times afterwards, for 3 weeks. Any louse able to move legs or antennae was considered alive and the goat recorded as infested. Results of these tests are summarized in table 1.

Table 1.—Relative effectiveness of dips of several chlorinated hydrocarbon insecticides for control of goat biting lice (10 goats per treatment).

Insecticide[a]	Per Cent Concentration	Number of Goats Infested After				
		1 Day	2 Days	1 Week	2 Weeks	3 Weeks
	Ranch 1					
Toxaphene (L)	0.25	1	7	0	0	0
Toxaphene (C)	.25	10	10	4	0	0
DDT (L)	.25	1	0	0	0	0
Toxaphene+lindane (C)	.09+.004	10	8	3	1	0
	Ranch 2					
Toxaphene (C)	0.25	10	10	10	10	10
DDT (L)	.25	10	2	0	0	0
Methoxychlor (L)	.5	7	0	0	0	0
Lindane (L)	.025	10	2	0	7	10

[a] L indicates laboratory formulations and C commercial formulations.

In the tests with goats from ranch 1 all insecticides were effective. DDT gave 100% kill in 48 hours, but the other materials required 1 to 2 weeks.

Lice on goats from ranch 2 were eradicated by the DDT and methoxychlor dips. Lindane gave excellent immediate control but reinfestations developed in 3 weeks, indicating a lack of residual effectiveness. Toxaphene provided very little control, indicating that the lice were resistant to this insecticide.

FIELD TESTS.—Extensive field tests were then conducted to evaluate the effectiveness of toxaphene and six other insecticides as sprays for the control of goat lice. These tests involved 23 flocks, totaling more than 6,000 animals. Flocks with different lengths of fleece were chosen. All flocks were heavily infested with biting lice but only one with sucking lice.

The insecticides used were Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Sevin® (1-naphthyl *N*-methyl carbamate), ronnel [Korlan grade], malathion, and toxaphene. The concentrations ranged from 0.1 to 0.5%, depending upon toxicity to animals and effectiveness in preliminary or screening tests.

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Table 2.—**Effectiveness of seven insecticide sprays for the control of goat biting lice in field tests.**

INSECTICIDE AND PER CENT CONCENTRATION	GALLONS USED	ANIMALS TREATED	TIME SINCE SHEARING	DAYS BEFORE COMPLETE KILL
Ronnel (25 WP)				
0.25	40	193	1 mo.	1
	150	594	2½ mo.	5–7
	75	280	Shorn	1
0.5	50	253	6 wk.	1
	100	394	5 wk.	1[a]
	100	422	Shorn	1
Delnav (30 EC)				
0.1	50	236	1 mo.	Incomplete[b]
	85	273	6 wk.	Incomplete
0.15	75	250	2 wk.	1
0.2	40	176	Shorn	1
Malathion (25 WP)				
0.1	100	220	Shorn	1
0.5	120	436	2 mo.	2–4
Dipterex (50 SP)				
0.15	50	184	2 wk.	1
0.25	125	545	2 wk.	1
	50	224	1 mo.	1
Bayer 21/199 (25 WP)				
0.1	50	227	1 mo.	1[a]
0.25	45	170	2 mo.	1
	45	169	2 mo.	1
	50	225	Shorn	1
	125	420	5 mo.	2–4
Toxaphene (50 EC)				
0.5	110	436	2 mo.	Incomplete[b]
Sevin (50 WP)				
0.25	100	511	2 wk.	1
0.5	125	460	5 mo.	5–7[a]

[a] Complete kill also at next shearing.
[b] Re-treated.

The insecticides were applied with a power sprayer at a pressure of 250 to 300 pounds per square inch. Each animal was treated with approximately 1 quart of spray. This amount was more than enough to wet thoroughly freshly shorn goats, but the degree of wetting of others varied with the length and density of the hair. Goats were sprayed in small groups to insure that each animal was treated.

Goats were examined for lice before treatment, after 24 hours, and at various intervals thereafter. Three flocks were examined at the next shearing, 2 to 4 months after treatment.

RESULTS.—Results of these tests are summarized in table 2. Bayer 21/199 and malathion at 0.1%, Delnav and Dipterex at 0.15% and Sevin and ronnel at 0.25% all gave complete kill in 1 day on goats freshly shorn or with 2 to 6 weeks' growth of hair. Control on goats with 2 to 5 months' growth of hair was somewhat slower, from 3 to 5 days, with the same or higher concentrations of insecticide. An application of 0.25% ronnel to the one flock infested with sucking and biting lice apparently gave complete control of the latter in 1 day, but 4 days were required for the sucking lice (data not shown in table). The standard treatment of 0.5% toxaphene failed to give satisfactory control in 5 to 7 days and the flock was retreated with malathion.

In a test with 0.2% Delnav, nannies were treated and after 4 hours returned to pens where their kids had been held. Subsequent observations showed that the nannies were free of lice but populations on the kids were not controlled. It was therefore indicated that control cannot be achieved without treating all the animals in a flock.

Three flocks that had been treated with 0.5% ronnel, 0.1% Bayer 21/199, or 0.5% Sevin were examined at the next shearing to ascertain whether the treatments had eradicated lice. All were free of lice.

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Nature of the Attractant in Sucrose Fed on by House Flies[1]

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ABSTRACT

The attractiveness to house flies (*Musca domestica* L.) of traps containing other house flies feeding on sucrose is probably related to a relative-humidity gradient generated by the moisture excreted by the flies. In a group of tests run at 50 to 73% ambient relative humidity flies responded to traps in which the relative humidity was 18 to 38% higher than the ambient value. The addition of about 6% of water to sucrose increased its attractiveness in petri-dish tests so that the mixture was usually more attractive than sucrose fed on by house flies. No evidence was found for a volatile chemical reaction product of sucrose having appreciable attractiveness. In the tests only 10 to 15% of the flies were attracted to fed-on sucrose, indicating that the fly factor is a low-order attractant.

The presence of an unknown attractant, or fly factor, in food baits such as cornmeal mush, proteose, and peptone that had been visited and fed on by house flies (*Musca domestica* L.) was demonstrated by Barnhart & Chadwick (1953). These investigators concluded that flies visiting a bait contribute to it some substance that makes it more attractive to the species.

From his experiments with black blow flies (*Phormia regina* (Meig.)) on the mode of action of sugar-baited fly traps, Dethier (1955) confirmed the existence of a fly factor. In addition, he concluded that it is not species specific and is composed of two volatile substances which might be identical—(1) a contaminant deposited on the glass funnel of the trap by escaping flies, and (2) material formed by a reaction between sucrose and something generated by the flies.

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Workers at the Orlando, Florida, laboratory have been attempting for some time to establish the nature of this fly factor because of its possible importance as an aid in the control of house flies, which are becoming increasingly resistant to a broad group of insecticides. These studies are described in this paper.

PRELIMINARY EXPERIMENTS.—Beginning in 1953, experiments were conducted (1) to extract an attractant from the atmosphere of the fly-rearing room, or from air bubbled through heated solutions of fed-on sucrose, by passing the air through traps containing 95% ethanol, and (2) to remove an attractant from fed-on sucrose by solvent extraction.[5] The liquid samples in drinking-glass traps closed with wire-mesh funnels, and the solid samples in petri dishes were tested for attractiveness in a 9- by 11-foot room stocked with 2,000 to 4,000 house flies from the Orlando regular colony. The data indicated that the flies chose certain test samples in preference to others, but no correlation appeared between the attractiveness of the samples and their mode of preparation.

In 1956 some of these experiments were repeated with slight modification. Air from the rearing room was drawn through 95% ethanol contained in gas-washing bottles for different lengths of time. In some tests the ethanol from the traps was distilled to a small volume at atmospheric pressure; in others it was distilled at 1 mm. pressure, completely or almost so in a closed system at room temperature. Tests of the samples of trap ethanol, of distillate, and of residue were made in the drinking-glass traps. The data given in table 1 show that the samples were usually more attractive than 95% ethanol, but not more than 50% ethanol. Again the data failed to correlate attractiveness with the length of the absorption period.

In another experiment house flies were allowed to feed on sucrose (2,000 flies per 50 grams for 2½ days) exposed in petri dishes or on metal foil. The fed-on sucrose was then ground and used at once or stored in a sealed container in a freezing chest. Portions were extracted with ether, and various combinations of the extracted fed-on sucrose, or marc, and the ether extract were compared with different standards by exposure in petri dishes after equivalent quantities of the liquids had been added to the solids and allowed to evaporate. Data presented in table 2 show the wide variation in activity sometimes encountered among samples from different lots of fed-on sucrose, the results being similar to those obtained previously when acetone and ethanol were used for extraction.

Table 1.—Attractiveness to house flies of ethanol extracts of air from the rearing room.[a]

DATE (1956)	DAYS EXTRACTION	ATTRACTANT	RATIO TO STANDARD
		Standard 95% ethanol (10 ml.)	
July 26	6	Residue (atmosphere)	13.3
30	3	Residue (vacuum, dry)	1.0
		Distillate	1.0
August 8	2	Trap ethanol	32.2
		Residue (atmosphere)	32.2
		Distillate	2.3
15	6	Trap ethanol:	
		10 ml.	11.0
		20 ml.	15.7
		40 ml.	5.7
		Residue (atmos.) 11.5 ml., equiv. 175 ml.	13.3
			3.3
21	6	Trap ethanol, 20 ml.	1.3
		Residue (atmos.) concentrated	
		10:1	2.2
		20:1	8.1
23	3	Residue (atmosphere)	19.0
24	9	Residue (vacuum, wet)	24.0
		Standard 50% ethanol (10 ml.)	
October 5	17	Residue (atmosphere)	1.2
		Residue (aged 4 weeks)	0.4

[a] Glass-trap tests.

Table 2.—Attractiveness to house flies of different samples of fed-on sucrose, their ether extracts, and their marcs.[a]

STANDARD	ATTRACTANT	RATIO TO STANDARD
Sucrose	Fed-on sucrose:	
	Lot A	1.9
	Lot B	23.8
	Ether extract of lot B, on marc	12.6
	Marc, lot B	12.7
Fed-on sucrose, lot A	Ether extract of lot A, on marc	2.5
	Marc, lot A	1.0
Sucrose plus ether	Ether extract of lot B:	
	On marc	0.6
	On sucrose	0.8
	Marc of lot B plus ether	0.9

[a] Petri-dish tests.

IMPROVED TEST PROCEDURES.—The need for a more controlled procedure for testing house fly attractants was apparent. Two procedures were developed.

Groups of 500 4-day-old Orlando regular-colony house flies under carbon dioxide anesthesia were selected 18 hours prior to the tests to allow for complete recovery. During this period the flies were offered a 10% honey solution. Then one group of flies was released in each of three screened cages (4-foot cubes) placed within a 9- by 11-foot room where the temperature was maintained at 79° to 84° F. The atmosphere of the room was circulated over porous firebricks placed in a pan of water so that the humidity was maintained at 50 to 73%. The flies were provided with drinking water and allowed to feed on sucrose for 30 minutes. One hour later the flies were again offered sucrose, and the tests were begun after 2% of the flies had fed.

Petri-dish Tests.—Four grams each of the unknown and of the sucrose standard in petri dishes were exposed in a cage. After 2 minutes the flies feeding on each sample were counted and disturbed from the dishes. Then the positions of the dishes were reversed and the samples were exposed for another 2 minutes. Four exposure periods constituted a test, and each test was replicated in two other cages. The ratio of the number of flies that fed on the unknown to the number that fed on the standard was calculated. Usually only 10 to 15% of the insects in a cage reacted to the samples.

Glass-trap Tests.—Four grams of the unknown was placed in a 12-ounce drinking glass and covered with wire mesh. A wire-mesh funnel having a ½-inch opening was placed in the mouth of the glass to complete the trap. The number of flies in this trap compared with those in

[5] Experiments conducted by J. C. Keller, H. G. Wilson, and P. L. Davis.

Table 3.—Effect of different amounts of water on the attractiveness of sucrose and fed-on sucrose to house flies.[a]

Standard	Per Cent of Water in Sucrose	Ratio to Standard
Sucrose	25.0	2.6
	12.5	3.5
	6.3	10.1
	3.1	8.1
	1.6	6.7
	0.8	5.3
Fed-on sucrose	6.3	3.6
Plus water		
3.1%	3.1	1.3
1.6%	1.6	1.5

[a] Petri-dish tests.

another trap containing a suitable standard was determined after 30 minutes in each of the cages. These test procedures were used in the following experiments unless otherwise noted.

Studies of Moisture Effects.—The lack of correlation in the results of the previous experiments cast some doubt as to the presence of a specific chemical attractant in the atmosphere of the rearing room or in fed-on sucrose. Therefore, a different nature of the fly factor was indicated. Obviously moisture could have been absorbed from the atmosphere by the trap ethanol, and the concentrated trap solutions could have contained more water than the original 95% ethanol. Moreover, moisture from the atmosphere could have condensed on the sucrose samples during the removal of the organic solvent by evaporation.

Therefore, tests were conducted to determine the relation of moisture to attractiveness. In one test 95% ethanol was less attractive in glass-trap tests than aqueous solutions containing 3 to 48% of ethanol; the lower concentrations were the more attractive. In another test 4 ml. of ethanol, acetone, or ether was mixed with separate 4-gram lots of sucrose and allowed to evaporate. The residues were then tested in petri dishes, and the following ratios of attractiveness to the sucrose standard were obtained: ethanol 5.83, acetone 3.67, ether 4.96. Then petri-dish tests were made on samples of sucrose to which 0.8 to 25% of water had been added, and the results (table 3) showed that the attractiveness of the sucrose increased to a maximum at 6.3% of water and then decreased as more water was added. It was also found that the lower concentrations of water were more attractive than fed-on sucrose either alone or with the same quantities of water added. Moreover, a round-robin petri-dish test in which each sample was compared with each other sample in a series (table 4), indicated the superiority of the sucrose-water mixture over fed-on sucrose.

From these results it was concluded that the fly factor is most likely an effect of moisture, and that fed-on sucrose is more attractive than sucrose because flies leave it in a better condition to maintain its moisture. The latter point was also indicated in drying experiments, when it was observed that fed-on sucrose absorbed moisture more readily and held moisture more tenaciously than sucrose.

Table 4.—Attractiveness to house flies of fed-on sucrose and sucrose plus water in a round-robin series of Petri-dish tests.

Attractant	Number of Flies: Range	Number of Flies: Adjusted Average	Ratio to Standard
Fed-on sucrose			
Lot A	2–49	23.9	2.1
Lot B	4–37	19.0	1.7
Sucrose			
Plus water 6.3%	7–46	32.7	2.8
Standard	9–25	11.5	1.0
L.S.D. at 5% level	—	10.9	—

The validity of this conclusion was tested by using the double-partition glass traps that led Dethier (1955) to draw his conclusions. Contrary to Dethier's finding that a double-partition trap was not effective, in these experiments it was found that the exposure of two such traps, each containing 25 flies feeding for 18 hours on 10 grams of sucrose, was very effective, since each caught 200 to 300 flies of the 2,000 placed in a rearing cage. After the flies had been removed from the traps, it was determined that the fed-on sucrose contained 7.8% of moisture.

To determine the relative effectiveness of traps that contained this quantity of moisture for flies feeding on sucrose, round-robin tests were run for 19 hours in cages stocked with 2,000 flies each. Table 5 shows that the traps containing 25 flies feeding on sucrose were no more effective than traps containing sucrose plus water, water on filter paper, or 25 flies alone. Therefore, it seemed unlikely that the fly factor was contamination or a reaction product of sucrose, but, instead, moisture operating in some unknown manner.

Effect of Relative Humidity.—To learn whether the fly factor might be identified with relative humidity, round-robin tests were made with glass traps in which the relative humidities were controlled through the use of aqueous solutions of sulfuric acid. Twenty ml. of the appropriate solution was added to each trap and allowed to equilibrate for 6 hours in the test cage stocked with 2,000 flies. The traps were then opened and the number of flies in each trap was determined after 2 hours. This shorter interval was used, since some parallel tests had shown that the final results were not unduly affected by it. These tests indicated that the flies preferred relative humidities

Table 5.—Attractiveness to house flies of sucrose, living flies, and water in a round-robin series in glass traps.

Attractant	Number of Flies: Range	Number of Flies: Adjusted Average	Ratio to Standard
Sucrose 10 grams, plus—			
25 Flies	28–243	100.8	0.4
Water 0.7 ml.	108–317	207.6	0.7
25 Flies	148–493	297.8	1.1
Water 0.7 ml. on filter paper	173–443	296.8	1.1
Standard, empty trap	134–355	281.2	1.0
L.S.D. at 5% level	—	89.4	—

Table 6.—Number of house flies attracted to glass traps adjusted to various humidities in a round-robin series.

Per Cent Relative Humidity	No Water in Cage		Water in Cage	
	Range	Adjusted Average	Range	Adjusted Average
100	4–68	27.5	0–17	6.4
90	10–46	25.9	1–17	7.8
80	1–81	23.8	1–17	6.0
70	1–51	17.0	0–11	5.7
60	1–32	12.2	0–10	3.6
L.S.D. at 5% level	—	7.9	—	2.5

of 60% and 80% to 20% and 40% but made little choice between 20% and 40% or between 60% and 80%.

In a repetition of these tests to cover the relative humidity range of 60% to 100% (table 6), the flies preferred the traps in which the relative humidity was greater than 80%. During these tests the average relative humidity of the room was 18 to 38% lower than that of the traps. When the drinking water was removed from the cages at the beginning of the tests, the flies showed a preference for higher humidities than when drinking water was available.

To learn whether the attractiveness of fed-on sucrose might be correlated with relative humidity, it was compared with sucrose equilibrated at 90% relative humidity for 6 hours. The samples were placed in the traps on pieces of cotton sheeting supported on the lower partition. As anticipated, both fed-on sucrose and sucrose equilibrated at 90% relative humidity were slightly better than the sucrose standard (table 7), but no significant differences between any of the samples were evident. Although these results indicated that fed-on sucrose contained nothing more attractive than moisture, the correlation of the fly factor with relative humidity was less conclusive than had been anticipated from the results of the previous experiments.

Drying Experiments.—In a further attempt to show the presence of an attractive reaction product of sucrose or some attractant other than water, 4-gram samples of fed-on sucrose obtained during the winter and early spring were oven dried to constant weight at 100° to 105° C. and atmospheric pressure. The moisture content of these samples differed (0.25%, 0.46%, and 0.66%), and they caramelized more or less during the drying process. However, the moisture content of samples of the sucrose standard differed also (0.13% and 0.28%). A quantity of water equivalent to the moisture the samples had lost on drying was then added to some of the dried fed-on sucrose, and then these samples were tested in petri dishes for attractiveness to house flies in comparison with the fed-on sucrose, the dried fed-on surcose, and the dried sucrose. Data given in table 8 show that the fed-on sucrose was more attractive than the sucrose standard. On the contrary, the dried fed-on sucrose was no more attractive than the dried sucrose, but the reconstituted samples of fed-on sucrose regained their superiority over the sucrose standard. These results again indicated that the only attractive material contained in fed-on sucrose was moisture.

Table 8.—Effect of drying on attractiveness of fed-on sucrose to house flies. Petri-dish tests.

Standard	Condition of Fed-on Sucrose	Ratio to Standard
Sucrose		
Undried	Undried	2.4
	Oven-dried plus water removed by drying	2.3
Oven-dried	Oven-dried	1.0
Fed-on sucrose		
Oven-dried	Vacuum-dried	1.2

In order to eliminate any effect of caramelization, fed-on sucrose obtained in the summer was dried at 80° C. and 1 mm. pressure. The moisture content of this fed-on sucrose was 0.10% and that of the sucrose standard 0.13%. The reconstituted samples of dried fed-on sucrose were not comparable with those used in the previous experiment owing to the difficulty of distributing the microquantity of water that had been removed. The results of two round-robin tests to compare these samples of fed-on sucrose before and after drying are shown in table 9. Fed-on sucrose was more attractive than the sucrose standard, but there was no significant difference between the fed-on sucrose before and after drying.

These results did not agree with those shown in table 8, which indicated the absence of an attractive reaction product of sucrose. No explanation for this discrepancy is apparent at present, because three tests showed practically no difference in attractiveness between oven-dried and vacuum-dried fed-on sucrose.

Discussion.—Dethier (1955) considered that water vapor might affect the attractiveness of baits. However, he tested only 0.01, 0.1, and 1.0 molar solutions of sucrose. Since the lowering of the vapor pressure by a

Table 7.—Attractiveness to house flies of fed-on sucrose and sucrose plus moisture in round-robin glass-trap tests.

Attractant	Number of Flies		Ratio to Standard
	Range	Adjusted Average	
Fed-on sucrose	2–78	32.2	1.4
Sucrose:			
Plus 6% water	2–37	21.4	0.9
At 80% relative humidity	0–64	19.3	0.8
At 90% relative humidity	3–108	28.2	1.2
Standard	4–57	23.4	1.0
L.S.D. at 5% level	—	10.4	—

Table 9.—Attractiveness of fed-on sucrose, before and after drying, to house flies in round-robin petri-dish tests.

Attractant	Number of Flies		Ratio to Standard
	Range	Adjusted Average	
Fed-on sucrose			
Before drying	75–111	80.0	2.6
Vacuum-dried	50–136	89.6	2.9
Vacuum-dried plus water removed on drying	59–135	89.9	2.9
Sucrose standard	7–23	30.5	1.0
L.S.D. at 5% level	—	29.1	—

nonvolatile solute is proportional to its mole fraction (Raoult's Law), the vapor-pressure lowering in the highest concentration was less than 3% which corresponds to about 97% relative humidity. Therefore, none of the three solutions that he tested gave a relative humidity below 97%. In addition, he reported that soiled funnels were attractive but rapidly lost their attractiveness on exposure. This effect may be due to moisture, since vomitus or excreta would be moist when freshly deposited but would lose their moisture to the atmosphere.

The possibility that feeding flies excrete an enzyme that converts sucrose to an attractant or that flies are attracted to their excreta has not been excluded. If this does occur, however, the attractant must have a specific activity much less than that of moisture or occur in insignificant quantity; otherwise, traps containing flies feeding on sucrose (table 5) would have been the most attractive on the assumption that the effects of the attractive forces are additive.

The activity of the flies diminished as the weather became warmer during the time the tests on the effect of humidity and drying were run. Although the decrease in activity did not appear to decrease the validity of these tests, the differences in attractiveness between the samples decreased as the weather became warmer. Moreover, the moisture content of fed-on sucrose was lower and differed very little from that of the sucrose standard.

The results of these experiments confirm some of the conclusions presented by Barnhart & Chadwick (1953) and Dethier (1955)—*e.g.*, the existence of a fly factor, its volatile nature, and its potential formation by the flies themselves. On the other hand, no evidence was found for the formation of such a factor by a reaction between sucrose and something generated by the flies. It is concluded that this fly factor is mostly moisture. *It seems evident that flies respond, not to moisture itself, but to the difference between the relative humidities generated by the samples undergoing test and the relative humidity of the surrounding atmosphere.* No evidence has been found of any other attractive material of appreciable potency in fed-on sucrose. Because of the small percentage of the insects that responded in the tests, it has also been concluded that the fly factor is a low-order attractant.

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Relative Efficiencies of Certain Spray Nozzles for Cotton Insect Control[1]

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ABSTRACT

Two conventional cone-type spray nozzles were compared with three kinds of wideswath, jet-type nozzles for effectiveness in controlling the boll weevil, *Anthonomus grandis* Boh., and the bollworm, *Heliothis zea* (Boddie). The wideswath nozzles were capable of spraying 6 to 12 rows per nozzle. Tests were arranged in randomized replicated small plots and were conducted under irrigated and dry-land conditions. Three rates of application per acre were used.

Results indicated that the conventional cone-type nozzles produced significantly better control of the boll weevil than the wideswath nozzles. They also were slightly more effective for controlling the bollworm. All treated plots produced significantly higher yields than the untreated check. However, the highest yields were harvested from the plots sprayed with the conventional cone type nozzles.

Card data collected for droplet size indicated that the wideswath nozzles produced larger droplets and decreased plant coverage with an increase in distance from the nozzle. Weevil mortality records and yield data indicated that this larger droplet size was mainly responsible for the inferior performance of the wideswath nozzles when compared with the cone nozzles.

Research has been conducted at the Texas Station for the past several years with regard to improving methods of insecticide application for control of cotton insects. Dean & Gaines (1950) compared spray and dust formulations of certain insecticides and found little difference between the two for control of the boll weevil, *Anthonomus grandis* Boh., and the bollworm, *Heliothis zea* (Boddie). Miller & Gaines (1951) reported that 1.8 gallons of spray per acre was as effective as 13.5 gallons for cotton insect control provided an equal amount of active toxicant per acre was used. Further work by Brown & Hanna (1954) confirmed these results, as they reported that about equally effective control of boll weevils and bollworms was obtained by timely applications of either 2, 6, or 14 gallons of spray per acre. The amount of toxicant per acre was held constant for the various gallonages. Smith & Hanna (1955) and Smith *et al.* (1956) summarized results of tests conducted during 1952–56 which indicated that cone-type nozzles were more effective for cotton insect control than fan-type spray nozzles. These workers also reported that nearly equal insect control and yields can be obtained with one cone nozzle per row delivering 2 gallons of spray per acre as with more costly and complex arrangements using two or three nozzles per row and delivering more spray per acre. Additional work by Magee & Davenport (1959) supported this earlier research, as they found sprays were equal to dusts, and that one cone nozzle per row delivering 2 gallons of spray per acre was as effective as arrangements using five nozzles per row delivering up to 30 gallons per acre. Results secured by these authors indicated that certain of the new

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jet-type, wideswath, boomless nozzles showed promise for cotton insect control. Results of experiments in which these nozzles were used in applying insecticides for control of the boll weevil and bollworm are reported herein.

DESCRIPTION OF NOZZLE TYPES AND ARRANGEMENTS.—Two arrangements of conventional cone-type nozzles were included in these experiments as standards for comparison. No. 6 cone nozzles spaced 40 inches apart on a 12-row sprayer boom were used for one arrangement. This arrangement placed one nozzle directly over each row. The No. 6 nozzle will deliver 7.2 gallons of spray per hour at 60 pounds of liquid pressure per square inch (p.s.i.). The other cone-nozzle arrangement utilized No. 3 cone nozzles spaced 20 inches apart on a 12-row boom. This arrangement positioned one nozzle over the row and one over the middle in an arrangement which made possible a blanket application along the length of the boom. The No. 3 nozzle will deliver 3.6 gallons of spray per hour at 60 p.s.i.

The wideswath jet-type nozzles did not require a sprayer boom for mounting. They were placed on special mounts at the rear of the sprayer, which could be lowered or raised as necessary in order to position the nozzles 36 to 40 inches above the tops of the plants. Three types of these nozzles,[3] Fieldjet, Floodjet, and Boomjet, were used. The maximum swath width of the Fieldjet and Floodjet was six rows, thus each nozzle was positioned over the third and fourth rows of the swath during operation. These nozzles were mounted in pairs in order to spray a 12-row swath with one pass of the machine. The Boomjet will spray a 12-row swath and this nozzle was positioned over the sixth and seventh rows of the swath during operation.

The Fieldjet nozzle will deliver 1.8 gallons per minute at 40 p.s.i. Spray from this nozzle was delivered at a wide angle from the nozzle orifice and was directed downward at approximately 45° to the ground line.

The Floodjet was similar to the Fieldjet except that spray was directed on a plane parallel to the ground line. This nozzle will deliver 1.7 gallons per minute at 35 p.s.i. The average spray angle at this pressure is approximately 160°.

The Boomjet, a single compact assembly containing five nozzle tips, was designed and positioned to deliver a spray pattern over a swath 38 to 66 feet wide. The nozzle used in these tests will deliver 3.5 gallons per minute over a 40-foot swath at 30 p.s.i.

EXPERIMENT 1.—Five spray-nozzle arrangements were tested in a randomized block design. Each treatment was replicated four times. Plots were 12 rows wide and 65 feet long. A 40-foot buffer zone was established at the ends of each plot in order to minimize effects of spray drift. Treatments applied were: (1) check, no insecticidal applications during the test period; (2) one No. 6 cone nozzle per row calibrated to deliver 6 gallons of spray per acre at 60 p.s.i.; (3) two No. 3 cone nozzles per row calibrated to deliver 6 gallons of spray per acre at 60 p.s.i.; (4) Fieldjet calibrated to deliver 6 and 10 gallons per acre at 32 p.s.i.; and (6) Boomjet calibrated to deliver 6 and 10 gallons per acre at 31 p.s.i. The desired amounts of spray per acre were obtained by varying the sprayer ground speed. For example, the 6-gallon-per-acre rate with the Floodjet was obtained by using a nozzle pressure of 32 p.s.i. and a ground speed of 7 m.p.h. To obtain the 10-gallon rate with this nozzle, the speed of the sprayer was decreased to 4 m.p.h.

Table 1.—Average seasonal insect infestation and yields of irrigated spray plots, College Station, Texas. 1958.

Nozzle Used	Gal./Acre	Per Cent Squares Injured by		Bollworm-Injured Bolls[a,c] (%)	Seed Cotton/Acre (Lb.)
		Weevil[a,b]	Bollworm[a,b]		
Check	—	42.1	15.1	21.6	493
1 No. 6	6	23.0	4.6	8.4	1,483
2 No. 3	6	29.5	8.0	8.0	1,381
Fieldjet	6	41.6	9.4	13.8	984
Floodjet	6	40.2	8.9	11.5	1,078
Boomjet	6	40.2	8.4	7.9	1,123
Fieldjet	10	40.2	8.5	14.2	1,103
Floodjet	10	37.4	10.3	9.7	1,351
Boomjet	10	41.2	10.8	10.0	1,071
L.S.D. at 5% level		6.7	2.7	4.3	141
at 1% level		9.1	3.6	5.8	191

[a] Per cents are expressed as angles.
[b] Based on seven counts (July 1 to Aug. 11).
[c] Based on three counts (Aug. 4 to 19).

This experiment was located in an irrigated field which had received the early-season insect control program as recommended in the Texas Agricultural Extension Service Guide L-218, "Texas Guide for Controlling Cotton Insects." Late-season applications made during the test period were predicated on insect counts. Treatment schedules varied somewhat from those recommended because of rainfall and irrigation practices. All plots received the same amount of actual toxicant per acre regardless of the schedule or gallonages per acre. Five applications of toxaphene-DDT were made at the rate of 2.0 pounds actual toxaphene plus 1.0 pound of actual DDT per acre per application. This mixture was applied July 2, 15, 21, 28, and August 6. Guthion-DDT at the rate of 0.3 pounds of actual Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate plus 1.0 pound of actual DDT per acre was applied on July 23, 31, August 11, and 16. Owing to an error, all plots were sprayed by airplane with 0.5 pounds of actual methyl parathion on July 9. All sprays were prepared from emulsifiable concentrates and were applied with a high-clearance ground sprayer.

Insect and yield records were taken from the four center rows of each plot. The plants reached a maximum height of approximately 5 feet during the growing season.

The percentages punctured squares recorded for the treatments, as shown in figure 1, indicated that the conventional low-gallonage, cone-type nozzle arrangements using either one nozzle directly over the row or one nozzle every 20 inches along the boom were most effective in controlling the boll weevil. The infestations in plots sprayed with the wideswath nozzles approached that recorded for the untreated check. The maximum infestation recorded for the check approached 80% punctured squares and furnished evidence of the degree of weevil pressure present in the experiment.

Seasonal average percentages of punctured squares recorded among the treatments were transformed to angles and subjected to the analysis of variance (table 1). These results indicated that the arrangements of one or two

[3] Furnished by the Spraying Systems Co., Bellwood, Illinois. The use of trade names in this paper is for identification purposes only and does not constitute an endorsement of the product by the Texas Agricultural Experiment Station.

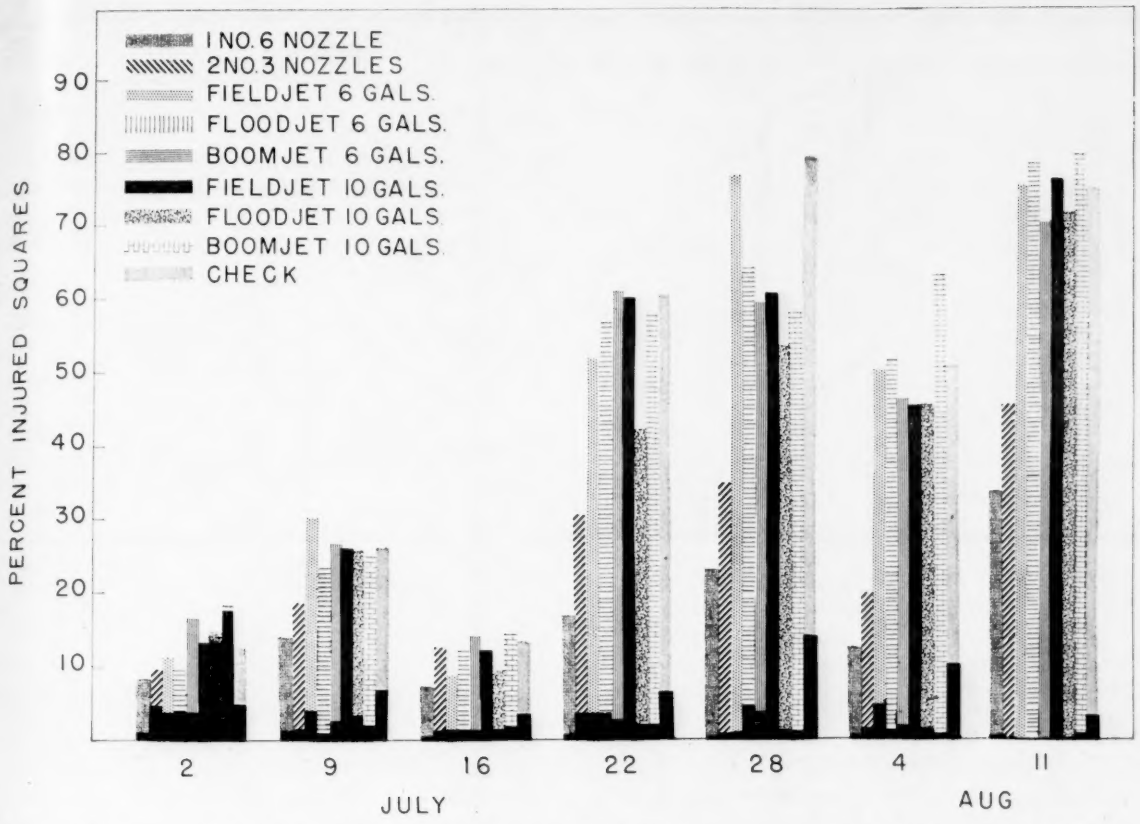

FIG. 1.—Weekly average boll weevil and bollworm square infestations recorded among various plots in Experiment 1. Bollworm infestations are illustrated by the solid black bars.

cone nozzles per row resulted in highly significantly better weevil control than the other nozzles. There were no significant differences in the boll weevil square infestations recorded among the wideswath nozzle and check plots.

Weekly bollworm-injured-square records indicated that all the nozzle arrangements produced some control of this insect (figure 1). Statistical analysis of the seasonal average bollworm square infestations (table 1) indicated that the one No. 6 cone nozzle per row treatment had significantly fewer injured squares than any of the plots. All the sprayed plots had significantly less bollworm square injury than the check. Records for percentage bollworm injured bolls (table 1) indicated highly significant more boll damage in the check than in the sprayed plots. Certain of the nozzle arrangements were slightly more effective than others.

Infestation records indicated that there was little difference in the control obtained between the 6 and 10 gallon rates of application with the wideswath nozzles.

Yields as reported in table 1 indicated that all the sprayed plots yielded considerably more cotton than the untreated check. The one- and two-cone-nozzle-per-row arrangements and the Floodjet at 10 gallons per acre produced yields significantly higher than harvested from plots sprayed with the other nozzles. These treatments resulted in gains of approximately 1,000 pounds of seed cotton per acre when compared with the check. Yields harvested from the Floodjet sprayed plots were higher than the insect control resulting from its use would indicate. No significant differences in yields between the 6- and 10-gallon rates occurred for the other wideswath nozzles.

EXPERIMENT 2.—The same general procedure was used in this test, as outlined in experiment 1 with regard to plot size, plot design, infestation records, spray machines, nozzle arrangements, yield records, et cetera. Exceptions will be noted below.

The same nozzle arrangements as tested in experiment 1 were used in this experiment. However, all nozzles were calibrated to deliver 9 gallons of spray per acre. The same pressures as indicated for each nozzle in experiment 1 were maintained, but ground speed of the sprayer was varied to obtain the desired dosage.

The experiment was located in a nonirrigated field. Frequent showers allowed the plants to produce rank growth and they reached a maximum height of approximately 6 feet during the test period. No insecticides had been applied to the field prior to this experiment. A mixture of 2.0 pounds actual toxaphene plus 1.0 pound actual DDT per acre was applied on July 16, 25, 30 and August 4. Guthion-DDT at the rate of 0.3 pound plus 1.0 pound of active ingredient, respectively, per acre was applied on August 8, 13, 18, and 21. A single application of 1.5 pounds of actual DDT per acre was applied on July 21. The check received no insecticides during the season.

Results, as presented in table 2, were comparable to

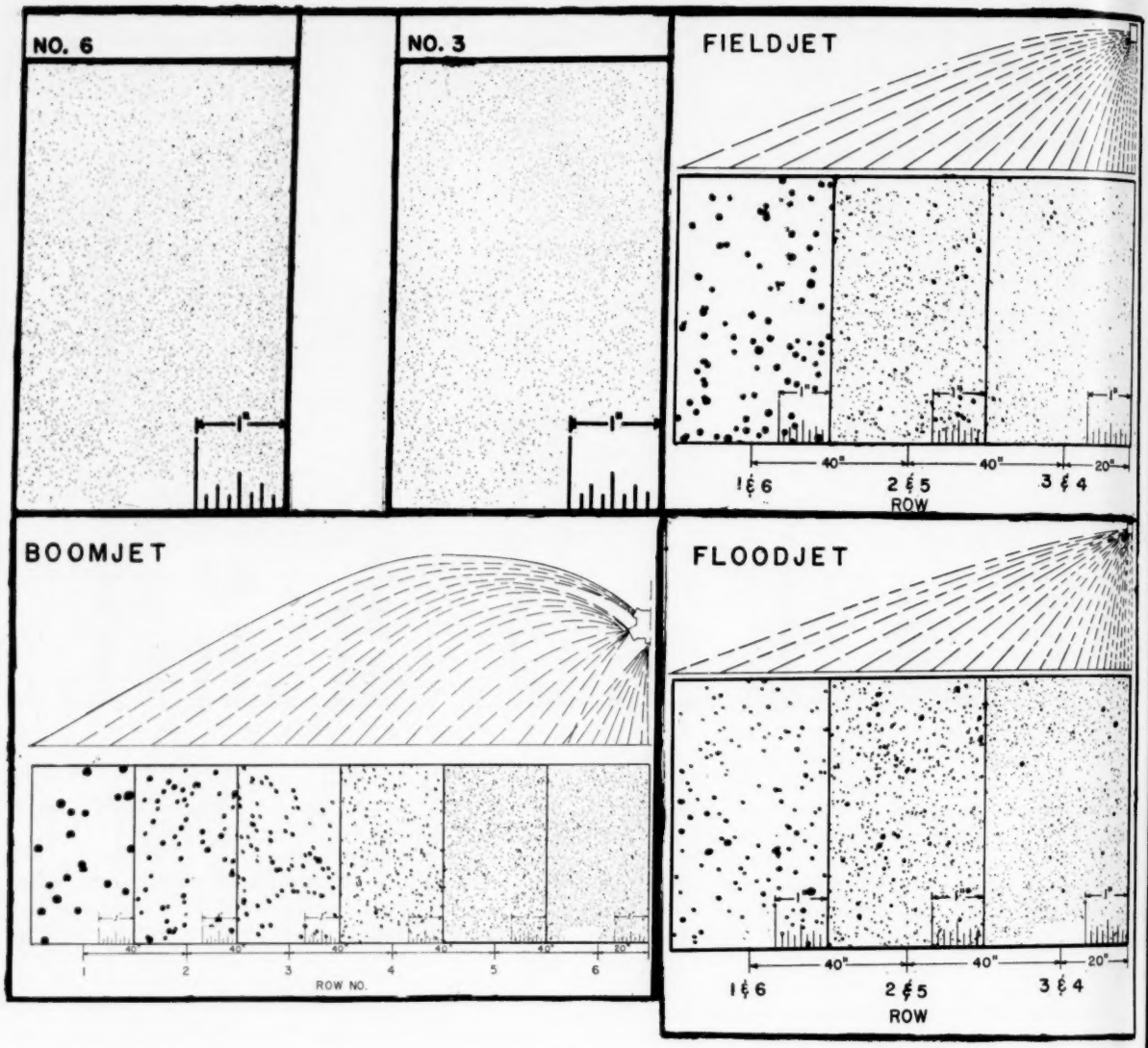

FIG. 2.—Photographs of spray droplets collected on 3×5-inch index cards when located various row distances from the nozzle.

those obtained in experiment 1. The use of conventional cone-type nozzles produced better control of the boll weevil and bollworm than the wideswath nozzles. The Floodjet did not perform so well in this test as in experiment 1. The boll weevil infestation recorded in the plots treated with this nozzle were slightly higher than recorded in the check. In this test the Boomjet was nearly as effective in controlling the boll weevil and the bollworm as the conventional nozzles.

Table 2.—Seasonal average insect infestations and yields of dryland spray plots, College Station, Texas. 1958.

Nozzle Used	Gal./Acre	Weevil-Punctured Squares (%)[a,b]	Per Cent Bollworm-Injured		Seed Cotton/Acre (Lb.)
			Squares[a,b]	Bolls[a,c]	
Check	—	35.0	12.3	19.2	1,120
1 No. 6	9	30.3	5.0	3.5	2,158
2 No. 3	9	29.2	5.5	4.3	1,951
Fieldjet	9	36.2	7.7	6.4	1,683
Floodjet	9	37.1	6.3	10.6	1,735
Boomjet	9	31.6	6.7	5.6	1,815
L.S.D. at 5% level		2.4	2.2	6.4	204
at 1% level		3.3	3.0	8.9	282

[a] Per cents are expressed as angles.
[b] Square injury based on seven counts (July 9 to Aug. 18).
[c] Injury based on three counts (August 4 to 19).

All the sprayed plots produced significantly more seed cotton per acre than the checks did, with gains of approximately 1,000 pounds resulting from the use of the most effective nozzles. The one- and two-cone-nozzle-per-row arrangements produced significantly more cotton than any of the other nozzles, except the Boomjet. The differences in insect infestations among the plots as reflected by the seasonal averages would not indicate such differences in yield. However, the plots sprayed with the conventional cone nozzles had lower weekly boll weevil square infestations than the other plots for all counts except the last two. At this time, a scarcity of squares and weevil migration into the experiment resulted in a high percentage of punctured squares in these plots and tended to bring the seasonal averages for all plots closer together.

Data presented for experiments 1 and 2 illustrate the consistency of control that can be obtained with the conventional cone-type nozzle arrangements. At the same time, the inconsistency of results obtained between experiments for the wideswath nozzles is pointed out.

It appears from the literature cited and from results of these experiments that cone-nozzle arrangements of one nozzle per row or nozzles spaced 20 inches apart on the boom will produce as effective cotton insect control as will the more complicated cone-nozzle arrangements, and better control than can be expected from the use of wideswath jet nozzles.

EXPERIMENT 3.—Early in Experiments 1 and 2 it became apparent that the wideswath nozzles were not so effective as the conventional cone nozzles. Therefore, observations were made as to the reasons for their ineffectiveness. Spray residue on the plants indicated that the droplet size produced by the wideswath nozzles progressively increased with distance from the nozzle orfices. That is, larger droplets were found on plants 3 to 6 rows from the nozzles than were present on plants of rows adjoining the nozzles. Also, it appeared that spray coverage of the plants decreased as droplet size increased. Thus, plants on the outer rows of the spray swaths of the wideswath nozzles did not receive as complete coverage as the plants on rows adjoining the nozzles or as the plants sprayed with the cone nozzles. Experiments were conducted in order to obtain data on these observations.

Specially built racks equipped with card holders capable of holding 3-×5-inch index cards were constructed of metal rods. These racks were attached to growing plants of each row in the spray swath for each nozzle. The index cards were attached in a position which simulated the upper leaves of the plants. The 3-×5-inch card was selected since its total area approximates that of the average sized cotton leaf. A carmine-type dye was added to the spray emulsion and card data on droplet size were thus collected under field conditions. The addition of dye to the spray made the droplet readily visible to the eye.

For the Fieldjet and Floodjet nozzles, card holders were located on rows adjoining the nozzle (rows 3 and 4) and two rows (rows 2 and 5) and three rows (rows 1 and 6) from the respective nozzles. For the Boomjet, card data were obtained for rows 1 through 6 with row 1 being farthest (six rows) from the nozzle and row 6 being the row adjoining the nozzle. Only one position was used for the cone nozzles, and that was a card holder located directly under the nozzles. The spray patterns collected on the various cards were photographed and are presented in figure 2.

Results indicated that the No. 3 cone nozzle produced the smallest size droplet, followed by the No. 6 cone nozzle. Both nozzles yielded complete and uniform coverage. The droplet size produced by each of the wideswath nozzles increased greatly as the distance from the nozzle was increased. From the card data, it appears clear that as droplet size increased plant coverage decreased. Droplets produced on rows closest to the wideswath nozzles were of an approximate size to those produced by the No. 6 cone nozzle and coverage was comparable. No data were obtained for density of droplets, but it appeared that the larger droplets were more dense than the smaller ones.

As the card data indicated considerable differences in the sizes of droplets produced along the spray swaths, cage tests were conducted to determine the effects on boll weevil control. Cages were constructed of pint ice cream cartons which had the original ends removed and screen disks inserted in the openings. The cages were placed on the cotton plants at various positions and distances from the respective spray nozzles as reported in table 3. Only one cone nozzle (No. 6, one per row) was used in this test.

Table 3.—Percentage mortalities of boll weevils caged on cotton plants at various locations and distances from several spray nozzles, College Station, Texas. 1958.

Nozzle Used	Cage Location 1[a]	2[b]	3[c]	4[d]	Mean
Check	6.2	25.0	7.5	7.5	11.6
1 No. 6	94.7	100.0	70.3	59.5	81.1
Fieldjet	89.5	96.7	8.1	29.7	56.0
Floodjet	85.5	93.3	24.3	10.8	53.5
Boomjet	93.4	53.3	8.1	29.7	46.1
Mean	73.9	73.7	23.7	27.4	—
L.S.D. at 5% level					30.7
at 1% level					43.0

[a] Weevils caged on plant terminals directly under nozzles.
[b] Weevils caged on plant terminals of swath row farthest distance from nozzles; under No. 6 nozzle, 3 rows from Fieldjet and Floodjet, and 6 rows from Boomjet.
[c] Weevils caged on same rows as b, except were caged midway down the plants. Cages were placed in middle toward the nozzles.
[d] Weevils caged on same plants and same positions as c, except cages were placed in opposite middles.

The cages were placed on the plants and the weevils installed within 2 hours after spraying with the Guthion-DDT mixture. Plants were approximately 4½ feet tall at the time of the test. Three- to five-day-old square-reared weevils were used in the tests. Ten weevils were installed in each cage and eight cages were used at each position. Comparable checks were installed in the untreated plots. Cages were allowed to remain on the plants for 24 hours, at which time they were removed and weevil mortality determined. Mortality was corrected by Abbott's formula (Abbott 1925). Results as presented in table 3 indicated that all the nozzles gave control of weevils caged on the plant terminals at all locations along the spray swath, with the exception of the Boomjet. The kill of weevils caged on the outer edge of the swath (6 rows from the nozzle) obtained by this nozzle was considerably below that recorded for the other nozzles. When weevils were confined in cages located midway on the plants, the control obtained by the wideswath nozzles was drastically reduced. This probably accounts for their inefficiency in keeping the percentage weevil-punctured squares at levels comparable with the cone nozzles. The means for mortality of all locations indicated the superiority of the cone nozzle, as it produced approximately 30% higher mortality than the wideswath jet nozzles.

Yield records were taken from each row of the plots treated with the wideswath nozzle. Comparable rows within each plot were paired and the yields combined, *i.e.*, yields of row 1 and 12 in the Boomjet plots were combined as these rows were comparable with regard to distance from the nozzle. Results for these nozzles are presented in figures 3, 4, and 5. These data indicated that yields per row decreased with distance from the nozzles

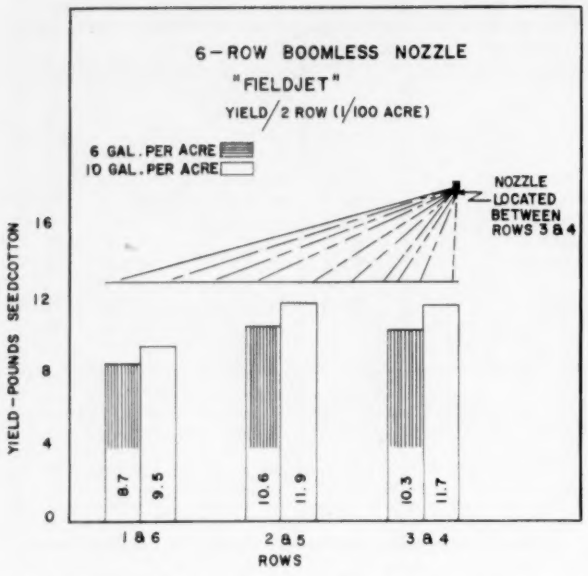

FIG. 3.—Pounds of seed cotton harvested from paired rows sprayed by the Fieldjet nozzle at 6 and 10 gallons per acre.

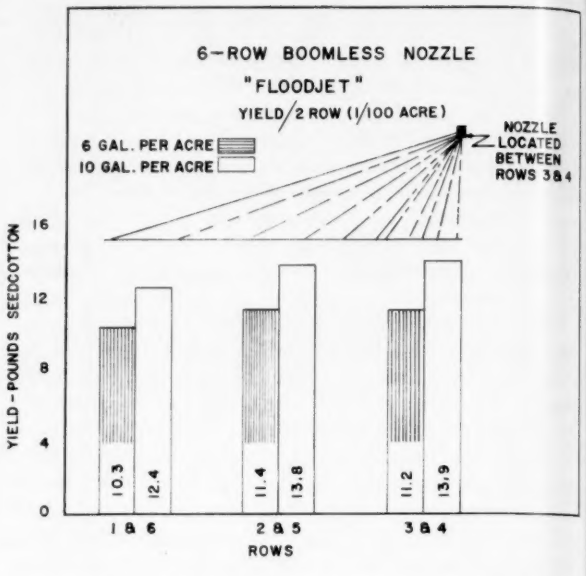

FIG. 4.—Pounds of seed cotton harvested from paired rows sprayed by the Floodjet nozzle at 6 and 10 gallons per acre.

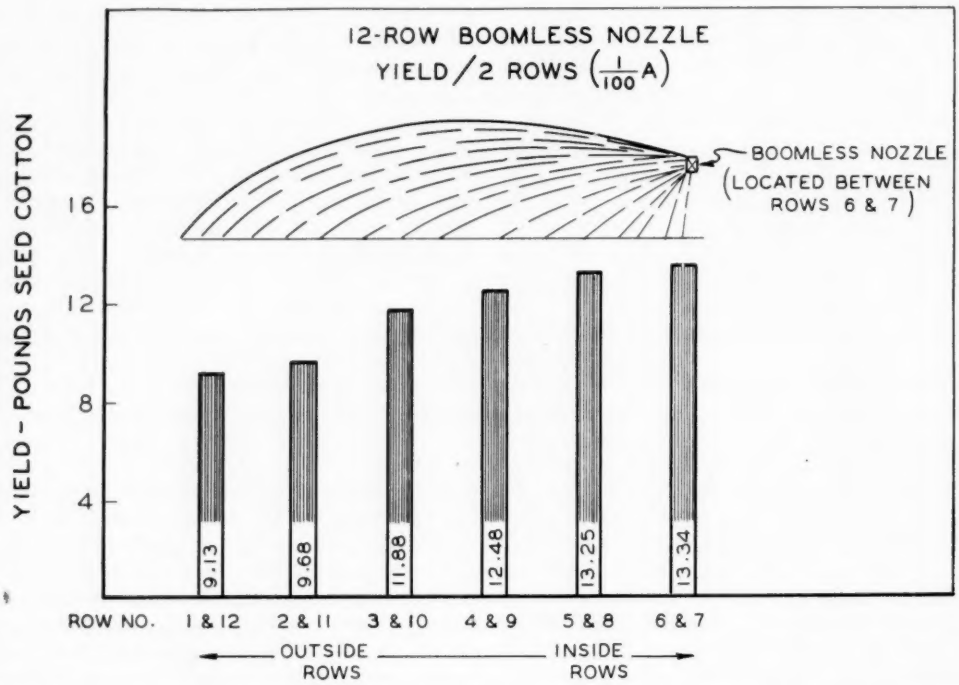

FIG. 5.—Pounds of seed cotton harvested from paired rows sprayed by the Boomjet nozzle at 6 gallons per acre.

as the rows toward the outer edge of the spray swath produced the least amount of cotton. Comparable yields were harvested from each row of the respective cone nozzle plots.

Thus, from data presented it appears that droplet size has an important effect on insect control. Mortality and yield records indicate that inefficiency of the wideswath nozzles for boll weevil control was largely due to (1) the large droplet size produced by these nozzles on the outer edge of the swath, and (2) the resulting poor spray coverage of the plants two or more rows distant from the nozzle.

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Insecticide-Induced Population Changes in Four Mite Species on Alfalfa[1]

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ABSTRACT

Three basic population effects were found resulting from spray treatments on alfalfa: (1) DDT treatments resulted in increased populations of the two-spotted spider mite *Tetranychus telarius* (L.), and reduced populations of *Typhlodromus cucumeris* Oudms. and *Tydeus* sp.; (2) Dieldrin and endrin increased populations of *Typhlodromus* but decreased *Tetranychus* and *Tydeus*; (3) Schradan and other organic phosphates increased populations of *Tydeus* and reduced *Typhlodromus* and *Tetranychus*. Combinations of materials caused intermediate reactions. A fourth species of mite, *Tarsonemus confusus* Ewing, abundant only during 1 year of the study, was increased in number by demeton and reduced by dieldrin.

The population changes occurred over a period of 4 to 6 weeks after treatment and the changes are the result of a combination of direct insecticide effects on individual species and of biotic interactions. Since large populations of *Typhlodromus* and *Tydeus* may develop in the near absence of two-spotted spider mites, these predators are not dependent on the two-spotted spider mite as a source of food.

During an investigation of the control of the two-spotted spider mite, *Tetranychus telarius* (L.),[3] and other pests of alfalfa grown for seed, it was found that populations of the predatory mite, *Typhlodromus cucumeris* Oudms., were low in some treatments and very high in other treatments. Further investigation showed that populations of *Tydeus* sp. and of *Tarsonemus confusus* Ewing also were affected, but in different ways.

Population studies on each of these mites have been continued in connection with insecticide investigations on alfalfa. The results of these investigations on insect control and alfalfa seed yield have been reported (Klostermeyer 1958). In each year of the investigation, 20 gallons of spray per acre were applied with a tractor-mounted sprayer using emulsifiable concentrate insecticides. Treatments were arranged in replicated randomized block designs of 3 to 5 replications. Plots usually measured 24′ by 65′. Usually the application was made just before bloom in early July. In most cases, a second application was made 3 to 4 weeks later. Mite populations on the foliage were determined by sampling each plot with 20 sweeps of a 15-inch diameter muslin sweeping net, anesthetizing the catch with carbon tetrachloride and separating the mites from the insects by shaking them through a 60-mesh screen with a specially constructed mechanical shaker. The mites were collected in small vials and brought to the laboratory, where the vials were emptied over a brushing machine onto a sticky glass plate. The mites on cross strips comprising $\frac{1}{10}$ of the plate area, (and thus $\frac{1}{10}$ of the mite population) were counted under a microscope. Details of this method were reported by Klostermeyer & Rasmussen (1956).

EXPERIMENTAL RESULTS.—The effects of the insecticides on the populations of typhlodromid mites were first noted in late summer of 1953. The populations of this and three other species of mites were determined on September 11, 1953, 4 weeks after the second spray application. Table 1 shows the population differences resulting from the 16 treatments. No untreated check was included in the experimental design. The data were transformed to the $\sqrt{n+0.5}$ for statistical analysis. This analysis showed highly significant differences among treatments for each of the mite species. In these 16 treatments, which combined 4 insecticides and 4 acaricides, both the insecticide portion of the combination and the acaricide acted on the population, and highly significant insecticide-acaricide interactions were noted, except with the *Tarsonemus* species. The acaricides, except for parathion, drastically reduced the population of two spotted spider mites. Only in the parathion combinations with other insecticides was the effect of the insecticide evident, and this primarily with DDT.

Both the acaricides and insecticides influenced the populations of *Typhlodromus* and *Tydeus*. In the case of the insecticide the effect was inverse to effects of the ma-

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[2] Wyatt Cone assisted with this work in 1956 and 1957 and W. B. Rasmussen assisted throughout the duration of the project.

[3] Samples of mites obtained in these studies were determined by E. W. Baker, Entomology Research Division, U. S. Department of Agriculture.

Table 1.—Effects of insecticide-acaricide combinations on four species of mites on alfalfa foliage, Prosser, Washington, September 11, 1953.

Acaricide	Insecticide				Total
	DDT	Toxaphene	Dieldrin	Heptachlor	
Tetranychus telarius per 10 sweeps					
Aramite	3	5	13	2	23
Aramite-parathion	3	5	7	3	18
Parathion	912	173	83	80	1,248
Demeton	0	1	4	5	10
Total	918	184	107	90	1,299
Typhlodromus cucumeris per 10 sweeps					
Aramite	32	185	352	300	869
Aramite-parathion	24	128	383	266	801
Parathion	54	103	209	227	593
Demeton	14	12	36	32	194
Total	124	428	980	825	2,357
Tydeus sp. per 10 sweeps					
Aramite	87	48	255	230	620
Aramite-parathion	30	136	332	364	862
Parathion	180	117	519	515	1,331
Demeton	103	185	569	830	1,687
Total	427	486	1,676	1,939	4,500
Tarsonemus confusus per 10 sweeps					
Aramite	89	54	52	118	313
Aramite-parathion	120	64	39	116	339
Parathion	91	78	36	120	325
Demeton	232	139	66	195	632
Total	532	335	193	549	1,609

terials on the two-spotted spider mites. DDT resulted in low population levels and dieldrin and heptachlor in high population levels. However, the acaricides affected the two species in a different manner. Demeton resulted in low populations of *Typhlodromus* and high populations of *Tydeus*.

In the case of *Tarsonemus confusus*, demeton appeared to increase the population and dieldrin to reduce it. In subsequent years the population of *Tarsonemus confusus* on alfalfa foliage was generally too low to evaluate with confidence.

Following these indications in 1953, mite counts were made in connection with other experimental work on insect control on alfalfa conducted in subsequent years (Klostermeyer 1958). Populations were sampled at weekly intervals after the first insecticide application. A total of 52 treatment combinations, each used in one or more years from 1954 to 1957, were evaluated. The general effects of these treatments, relative to the untreated checks, are indicated in table 2.

Three basic population effects were noted: (1) *Tetranychus* was increased, while *Typhlodromus* and *Tydeus* were reduced, illustrated by the DDT treatment; (2) *Typhlodromus* increased and other species decreased or were unaffected as shown by the dieldrin treatment; (3) *Tydeus* increased and other species decreased as shown by schradan and other organic phosphate treatments. Combinations of these materials caused intermediate reactions.

The effects of the materials on the seasonal cycle of the mites are indicated in figure 1 for data obtained in 1956. A second application made in some years generally intensified the effect of the first application.

Table 2.—Effects of insecticide treatments during 1954 to 1957, relative to the untreated checks, on mites.

1st Application	2nd Application
Tetranychus increased, other species decreased	
DDT	None
DDT	DDT
DDT-Isolan®[a]	DDT-Isolan
DDT	Toxaphene
DDT-schradan	DDT
Dieldrin	DDT
Toxaphene	Toxaphene
Tetranychus and *Typhlodromus* increased, *Tydeus* decreased	
Dieldrin	Toxaphene
Dieldrin-Isolan	Dieldrin-Isolan
Endrin	DDT
Endrin	None
DDT-parathion	None
Tetranychus decreased, *Typhlodromus* increased, *Tydeus* decreased	
Dieldrin	None
Dieldrin-Aramite	None
Dieldrin-Chlorobenzilate®[b]	None
Dieldrin-parathion	None
Heptachlor	None
Dylox®[c]	Dylox
Toxaphene	None
Toxaphene-Aramite	Toxaphene-Aramite
Toxaphene-parathion	Toxaphene-Aramite
DDT-Aramite	None
DDT-demeton	DDT-demeton
Tetranychus decreased, *Typhlodromus* and *Tydeus* increased	
Toxaphene-demeton	None
Dieldrin-Shell OS 1808[i]	Dieldrin-Shell OS 1808
Parathion	None
Parathion-Aramite-endrin	Aramite-DDT
Tetranychus and *Typhlodromus* decreased, *Tydeus* increased	
Demeton	Demeton
Dieldrin-schradan	Dieldrin-schradan
Schradan	None
Guthion®[e]	None
Diazinon®[f]	None
Diazinon	Diazinon
Trithion®[g]	None
Trithion	Trithion
Trithion-endrin	Trithion-DDT
All species decreased	
Demeton-endrin	DDT
DDT-demeton	DDT-demeton
Dieldrin-demeton	Dieldrin-demeton
Diazinon-DDT	DDT
Diazinon-DDT	None
Diazinon-endrin	Diazinon-endrin
DDT-schradan	DDT-schradan
Trithion-DDT	DDT
DDT-parathion	DDT-parathion
DDT-parathion	DDT
DDT-parathion	Toxaphene-Aramite
Aramite-DDT	Aramite-DDT
DDT-Compound 1808	DDT-Compound 1808
Dylox-DDT	Dylox-DDT
Aramite	Aramite
Parathion-Aramite	Aramite

[a] Dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate.
[b] Ethyl 4,4′-dichlorobenzilate.
[c] *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.
[d] 2-carbethoxy-1-methylvinyl diethyl phosphate.
[e] *O,O* dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate.
[f] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[g] *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.

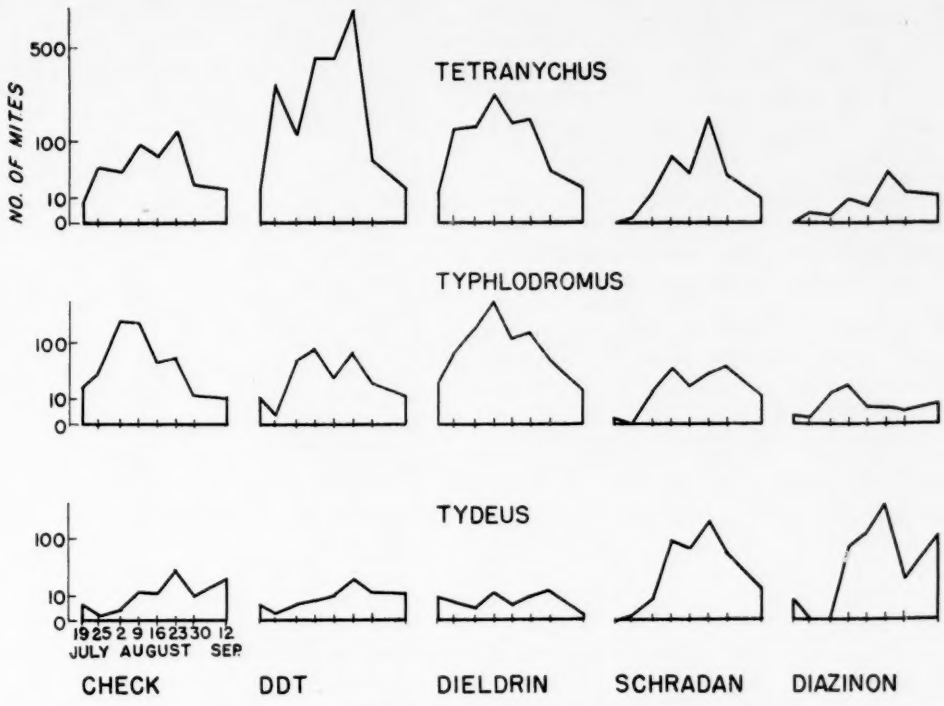

FIG. 1.—Changes in mite populations on alfalfa following insecticide applications on July 5 and Aug. 13, 1956, Prosser, Washington. Populations per six sweeps are plotted on a square-root scale.

Mite populations varied from year to year. This fact was particularly evident with *Tetranychus*, as shown in table 3. Populations were low in 1954, 1956, and 1957, but high in 1955. The populations of *Typhlodromus* were more or less the reverse of those of *Tetranychus*, while *Tydeus* populations had a seasonal variation similar to *Tetranychus*. *Tarsonemus* was abundant only in 1956.

DISCUSSION.—The population changes indicated here are those that occur on the alfalfa foliage. It had been noted that only adult *Typhlodromus* were present on the foliage although both adult and nymphs of *Tetranychus* and *Tydeus* were found. In 1957 Wyatt Cone found *Typhlodromus* and *Tydeus* in the plant debris on the soil surface beneath the plants. All stages of *Typhlodromus* were present there. It was also learned that *Typhlodromus* adults migrate to the foliage during the night and return to the soil during the day. The soil surface has a large fauna of mites and insects. The inter-relationships of the complex soil fauna and foliage fauna have not been worked out, nor are the food relationships of the predatory species *Typhlodromus* and *Tydeus* completely known. Chant (1958) states that typhlodromid mites can develop on fungal spores or plant pollen—prey in the form of mites not being necessary for survival. Brickhill (1958), in a study of *Tydeus bakeri* Brickhill and *Lorryia ferulus* Baker, found that both species could be reared on natural or synthetic honeydew. They also developed on two-spotted spider mite eggs but did poorly on other mite or insect eggs.

Table 3.—Seasonal differences in mite populations on alfalfa, Prosser, Washington, 1954–57.

MITE GENUS	NO. OF MITES PER 10 SWEEPS[a]			
	1954	1955	1956	1957
	Untreated			
Tetranychus	93	364	94	6
Typhlodromus	60	45	121	137
Tydeus	48	207	24	18
Tarsonemus	3	35	9	—
	2 lbs. DDT/Acre			
Tetranychus	254	632	443	25
Typhlodromus	66	38	70	118
Tydeus	14	134	16	2
Tarsonemus	3	65	3	—
	0.5 lb. Dieldrin/Acre[b]			
Tetranychus	67	28	225	—
Typhlodromus	134	132	170	—
Tydeus	21	61	12	—
Tarsonemus	0	10	2	—

[a] Means of 8 weekly samples in 1954, 5 in 1955, 8 in 1956 and 10 in 1957.
[b] With 2 lb. Chlorobenzilate in 1955.

The results reported here primarily concern the population changes occurring over a period of weeks after insecticides are applied. Hence, these changes are the result of a combination of direct insecticide effects on individual species and of biotic interactions. The relative influence of the two factors has not been determined. Evaluation of the direct and immediate toxicity of insecticides to each mite species is underway. Populations of most species of mites were depressed immediately after treatment, but biotic factors evidently come into play very quickly because increases in mite populations often are noted within a week of application.

Particularly puzzling are the effects of schradan and other systemics. These materials exert a severe depressing effect on *Typhlodromus* while at the same time greatly

increasing populations of *Tydeus*. Evidently the toxicity of schradan to *Typhlodromus* is greater than for the other predators cited by Ripper *et al.* (1951) who indicated little or no toxicity of schradan for predators. Since other nonsystemic, organic phosphates have a similar influence on the populations of *Typhlodromus* and *Tydeus*, the effect is evidently characteristic of this class of insecticides.

These observations are of little direct practical application to problems of insect and mite control in alfalfa seed production. The population of the pest species, the two-spotted spider mite, is not usually held in check by the predator *Typhlodromus cucumeris*, although the increase in two-spotted spider mites following DDT applications may be due in part to destruction of *Typhlodromus* by DDT. However, as shown by the dieldrin-Aramite (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) treatment in table 1, populations of *Typhlodromus* can become very large in the near absence of two-spotted spider mites, indicating that these are general predators, not dependent on two-spotted spider mites for food.

The results obtained during this 5-year study demonstrate the complex of biological changes that can be expected in insect and mite populations from insecticide treatment in even a very limited and uniform environment, such as an alfalfa field.

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Grass Hosts of Cereal Aphids[1]

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ABSTRACT

The relative host efficiency of 59 grass species in 30 genera are given for the English grain aphid (*Macrosiphum granarium* (Kirby), and the apple grain aphid (*Rhopalosiphum fitchii* (Sand.). Adult and nymphal survival and rate of reproduction varied with aphid species and with plant species within a genus. The most efficient hosts for the English grain aphid were *Festuca rubra, F. arundinacea, F. ovina* and *Lolium multiflorum;* for the apple grain aphid were *Agropyron poponi, Bouteloua arvensis, Elymus junceus, Festuca arundinacea, F. rubra* and *Lolium perenne.*

Records of aphid trapping at Centre Hall, Pennsylvania, have shown the English grain aphid, *Macrosiphum granarium* (Kirby), and the apple grain aphid, *Rhopalosiphum fitchii* (Sand.), to be abundant cereal aphids in the field. Both species are vectors of the barley yellow-dwarf virus (BYDV) of cereals. They feed on small grains during most of the growing season and migrate to alternate grass hosts during the summer. Many of these summer hosts are reservoirs of BYDV and hence are very important in the vector-virus-host relationship.

MATERIALS AND METHODS.—English grain aphids and apple grain aphids were confined on individual leaves of 59 grass species in 30 genera (table 1) in the greenhouse operating at approximately 68° F. and 70% relative humidity. The grass plants were grown in individual 4-inch plastic pots and were approximately 1 year old at the time of use. Three cages were used to confine single apterous adult aphids of each species on individual grass leaves.

The cages were made from $\frac{1}{2}'' \times 3\frac{1}{2}''$ #655 Lusteroid centrifuge tubes with the closed end removed. Each tube was clamped in the jaws of a wooden snap-type clothes pin. A $\frac{1}{8}'' \times \frac{1}{2}'' \times 12''$ wooden stake, pushed into the pot soil, was forced between the opposite ends of the clothes pin at the desired height. The cage was held in position by the pressure of the clothes pin on the stake.

The cage was positioned directly over a randomly selected leaf. The leaf was inserted into the tube through the lower opening, which was then plugged with cellucotton. This material did not interfere with adult or nymphal movement within the cage as long-fibre cotton plugs did in earlier tests.

Six days after aphid introduction, records were made of adult mortality, number of nymphs produced, and number of dead nymphs. Observations were made through the wall of the transparent cages.

DISCUSSION AND RESULTS.—The rate of reproduction and the survival of progeny on a given host are important measures of host efficiency. The ability of aphid progeny to survive rests, in part, upon the presence of required nutrients in the cell sap of the host during the period of infestation.

When feeding stimuli have been satisfied and the required nutrients are present in the sap, normal feeding should occur and be conducive to normal development. This contingency implies high progeny survival under optimum physical conditions. However, in a discussion of feeding by phytophagous insects, Thorsteinson (1958) made the following statements: "Normal development implies normal feeding, but normal feeding is not sufficient for normal development," and "normal development implies normal feeding on a diet that includes both sapid and vapid nutrients."

In cases where aphid progenies continue to feed and

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Table 1.—**Relative host efficiency, total progeny, nymphal and adult survival of the English grain aphid and the apple grain aphid at the end of 6 days' confinement on 59 grass species in the greenhouse.**

Grass Species	English Grain Aphid				Apple Grain Aphid			
	Per Cent Survival		Total Progeny	R.H.E.[a]	Per Cent Survival		Total Progeny	R.H.E.[a]
	Nymph	Adult			Nymph	Adult		
Agropyron elongatum	10	0	10	1	100	67	8	8
A. poponi	17	33	6	1	84	67	38	32
A. pungens	0	0	6	0	100	0	2	2
A. riparium[b]	50	0	8	4	100	67	7	7
A. trichophorum[c]	47	33	17	8	99	100	20	20
Agrostis alba[b,c]	7	0	14	1	33	33	9	3
A. tenuis[b,c]	0	0	14	0	18	0	11	2
Alopecurus smithii	44	67	9	4	100	67	5	5
Andropogon armulatus	6	33	17	1	11	100	28	3
A. intermedium	0	0	7	0	0	0	0	0
A. ischaemum[b,c]	65	33	23	15	29	100	27	8
A. saccharoides	17	0	6	1	8	67	37	3
Arrhenathorum elatius[b,c]	0	33	12	0	17	0	6	1
Axonopus affinis[b,c]	0	0	0	0	33	0	9	3
Bouteloua arrensis[b,c]	25	0	16	3	100	67	33	33
B. curtipendula	18	0	11	2	100	0	1	1
B. hirsuta	0	33	5	0	100	100	4	4
B. inermis[c]	0	0	12	0	8	100	25	2
Buchloe dactyloides[b,c]	12	67	16	2	75	0	8	6
Chloris gayana	28	100	13	4	23	0	13	3
Elymus giganteus	43	0	14	6	39	33	18	7
E. junceus	85	67	13	11	72	100	36	26
Eragrostis chloromelas	0	33	13	0	91	100	22	20
E. curvula	0	33	13	0	47	67	34	16
E. lehmanniana	0	0	3	0	0	0	0	0
E. trichodes	36	0	11	4	56	100	32	18
Festuca arundinacea	92	67	26	24	93	100	43	40
F. ovina[b,c]	92	67	25	23	100	33	11	11
F. rubra[b,c]	97	100	33	32	100	67	27	27
Hilaria jamesi galleta	43	33	7	3	100	33	4	4
Holcus lanatus[b,c]	0	0	9	0	50	0	2	1
Hordeum bulbosum	89	33	19	17	100	67	8	8
Lolium multiflorum[b,c]	89	100	27	24	91	100	23	21
L. perenne[b,c]	35	67	23	8	99	100	57	56
Muhlenbergia wrightii	0	0	8	0	100	100	16	16
Oryzopsis miliacea	0	0	6	0	6	67	18	1
Panicum antidotal	13	33	23	3	0	0	30	0
P. obtusum	0	0	6	0	0	0	0	0
P. virgatum	20	0	10	2	0	0	6	0
Paspalum dilatatum	0	33	2	0	0	0	0	0
P. notatum	0	67	7	0	—	—	—	—
Pennisetum arundinacea	8	0	12	1	—	—	—	—
P. ciliare	0	33	10	0	15	0	20	3
P. glaucum[b]	86	33	7	6	95	100	21	20
Phleum boehermi	11	33	21	2	60	0	2	1
Poa ampla	11	0	9	1	60	0	5	3
P. annua[b,c]	31	100	13	4	64	0	11	7
P. neradensis	0	33	9	0	100	33	5	5
P. pratensis[b,c]	22	33	23	5	44	33	9	4
P. trivialis[c]	87	67	15	13	50	0	4	2
Sorghastrum nutans	0	0	1	0	0	0	0	0
Sorghum almum	20	33	10	2	75	0	4	3
S. halepense[c]	100	67	14	14	100	0	3	3
S. sudanense	69	67	13	9	100	67	12	12
Sporobolus giganteus	7	0	15	1	62	67	8	5
Stipa viridula	93	33	14	13	100	67	9	9
Trichachne californica	0	0	11	0	5	33	18	1
Trisetum flavescens	83	67	18	15	86	67	21	18
Avena sativa—Check	100	100	44	44	100	100	32	32
Average	30.6	32.3	13.2		59.6	45.0	15.1	

[a] Relative host efficiency (see text for explanation).
[b] Previously recorded as host of English grain aphid.
[c] Previously recorded as host of apple grain aphid.

mature with little or no mortality, one can assume that the necessary sapid and vapid nutrients are present and that the plant is a satisfactory host for the species. When progeny have a high rate of mortality on a host, one or more of these nutrients might be absent, a toxic or repellent substance might be present in the sap, or the physical condition of the host may interfere with the required feeding procedure or stimuli.

Grass hosts of cereal aphids are an important ecological factor in grain production because of their direct bearing on aphid populations, their general susceptibility to BYDV, and their roles as summer reservoirs of BYDV, which is transmitted from them to small grains in the spring and fall. These factors make it important to know the host efficiency of each grass in maintaining populations of aphids that are virus vectors.

The efficiency of a grass species in maintaining an aphid population depends upon (1) the ability of the adult aphid to obtain required nutrients from the host over a period long enough to produce progeny, (2) the ability of the progeny to feed and mature on the host, and (3) the satisfaction of stimuli necessary to result in reproduction.

The relative host efficiency for 59 grass species, table 1, has been obtained by multiplying the percentage of nymph survival by total progeny (at the end of 6 days) and dividing by 100. The calculated value is not dependent upon a check response but can be compared with a preferred host, Garry oats (*Avena sativa*) in these experiments, for a general efficiency comparison. The importance of a given grass in the relationship between the aphid vector population and the incidence and potential spread (or dissemination) of BYDV is directly proportional to the relative host efficiency of that grass. When all nymphs died on the plant or when reproduction did not occur, the relative host efficiency became zero. This condition indicated a host completely unsatisfactory for the aphid species.

Even though a host fulfills all necessary physical and physiological stimuli for reproduction, the relative host efficiency becomes zero if these progeny suffer 100% mortality. Zero relative host efficiency also results when the reproductive stimuli are lacking, even though the host supplies the needed nutrients for progeny growth.

Host-plant lists for aphids include those plants from which aphids have been collected. These lists may not stipulate that the recorded host is one upon which feeding and reproduction are efficient or even occur. Therefore, one cannot use all aphid-host lists without questioning host efficiency.

Table 1 includes species that have been recorded as hosts for the two aphids of this test. The data substantiate the suitability of certain of these grasses as hosts and indicate that others are very poor hosts or that aphid feeding or reproduction cannot occur upon them.

Normal aphid development has not been evaluated in these tests, but normal and abnormal feeding is indicated by the percentage survival of the progeny and adults (table 1). Survival on grass species ranged from 0 to 100% for both aphid species. Average nymphal survival on the 59 grasses was 30.6 and 59.6% for the English grain and apple grain aphids, respectively. Average adult survival was also higher for the apple grain aphid.

Nymphal survival on grass species within the genera tested showed great variation in some cases and was quite uniform in others. Nymphal survival on *Festuca* spp. was uniform and high for both aphid species. Survival on *Bouteloua* spp. ranged from 0 to 25% for the English grain aphid and from 8 to 100% for the apple grain aphid.

Assuming the presence of essential sapid and vapid nutrients in the *Festuca* species tested to account for the high survival, species of *Bouteloua* would appear to vary greatly in the presence of these essential nutrients, or to contain toxic materials detrimental to survival. These data point to the need for testing individual grass species for host suitability and to the danger of assuming that because of high survival on one species, all species of that genus will be satisfactory hosts.

The host efficiency of a given grass species for different aphids may also vary greatly. Nymphs of the Englsh grain aphid failed to survive on *Muhlenbergia wrightii*, while nymphs of the apple grain aphid had 100% survival. Survival of adults on the grasses tested followed variations similar to those described for nymphs.

The total number of nymphs (progeny) produced during the 6 days of testing varied greatly among the grasses for both aphid species. The highest progeny number for the English grain aphid was 33 on *Festuca rubra*. This was 75% of the progeny number on oats, *Avena sativa*, var. Garry used as a check host. The maximum progeny produced by the apple grain aphid was 57 on *Lolium perenne*, and this figure was 178% of the progeny on oats.

As has been mentioned, the number of nymphs produced on grass species within a genus was not uniform, and the number of progeny produced by one aphid species could not be used to indicate the number of progeny of the other species. The greatest progeny number for the apple grain aphid occurred on *Lolium* (57), *Festuca* (43), *Agropyron* (38), *Andropogon* (37), and *Elymus* (36).

By employing the relative host efficiency rating as presented in table 1, these data indicate that *Festuca arundinacea*, *F. ovina*, *F. rubra*, and *Lolium multiflorum* are the most efficient hosts of those tested for the English grain aphid. Host efficiency appeared more widespread with the apple grain aphid, however, and for this aphid the grasses *Agropyron poponi*, *Bouteloua arvensis*, *Elymus junceus*, *Festuca arundinacea*, *F. rubra*, and *Lolium perenne* were the most efficient hosts. Other grass species may also be important hosts of these two aphids.

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The Metabolic Fate of Prolan in a Dilan-Resistant Strain of House Flies[1]

ALBERT S. PERRY and ANNETTE J. BUCKNER[2]

ABSTRACT

A susceptible laboratory strain of house flies (*Musca domestica* L.), exhibited resistance to Dilan (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts (1,1-bis(*p*-chlorophenyl)-2-nitrobutane) after several generations of selection pressure with that insecticide. Resistance to the Prolan® (1,1-bis(*p*-chlorophenyl)-2-nitropropane) component of Dilan was much more pronounced than that to the Bulan® (1,1-bis(*p*-chlorophenyl)-2-nitrobutane) component or to Dilan.

Metabolism tests showed that 75 to 94% of the 8 micrograms of Prolan applied was recovered from 2 to 120 hours after topical treatment of female resistant flies. At 24 and 48 hours, 42.5 and 62.8% of the dosage applied was absorbed by the insect. Two compounds were detected in the excreta of treated flies: (1) A neutral material, soluble in common organic solvents, similar to prolan in infrared, ultraviolet, and colorimetric spectra, and almost as toxic to mosquito larvae as the parent compound, and (2) an acidic material, extractable with dilute alkali, different from Prolan in photometric spectra and chemical properties (showing loss of NO_2 group in the propane moiety), and much less toxic to mosquito larvae.

Excretion of the toxic metabolite appeared to be more important to the fly's survival in the initial stages of poisoning.

The insecticide Dilan (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts (1,1-bis(*p*-chlorophenyl)-2-nitrobutane) which consists of 1 part Prolan® (1,1-bis-(*p*-chlorophenyl)-2-nitropropane) and 2 parts Bulan® (1,1-bis(*p*-chlorophenyl)-2-nitrobutane) was first synthesized by Hass *et al.* (1951). Although initially Dilan showed some promise in the control of DDT-resistant house flies in field and laboratory tests (March & Metcalf 1952), its potential usefulness against this species was considered limited since Dilan-resistant strains were produced under selection pressure with this chemical. Starting with a house fly (*Musca domestica* L.) strain which was resistant to a mixture of chlorinated hydrocarbon insecticides, Bruce & Decker (1951) were able to produce a Dilan-resistant strain after five generations of selection pressure with this insecticide.

The studies of Fullmer & Hoskins (1951) showed that susceptible flies respired at a much higher rate than a DDT-resistant strain following topical application of DDT, but essentially no difference appeared in the rate of respiration of the two strains when Dilan was applied, thus equal susceptibility of the two strains to this chemical was inferred.

At this laboratory, a Dilan-resistant strain was obtained by subjecting the susceptible NAIDM (National Association of Insecticide and Disinfectant Manufacturers) strain for several generations to selection pressure with residual applications of Dilan on plywood panels. Using topical applications of the pure chemicals, preliminary tests revealed that resistance to Prolan was much more pronounced than that to Bulan or Dilan. This report covers, for the most part, the results of investigations on the metabolic fate of Prolan in the resistant strain.

MATERIALS AND METHODS. Standard grade Prolan, m.p. 83.5–84.5° C., Bulan m.p. 64–65° C. and Dilan, m.p. 62–64° C., used throughout this investigation, was obtained through the courtesy of Commercial Solvents, Inc., Terre Haute, Indiana. Exploratory tests on suitable analytical methods for the recovery of Dilan and its components indicated that the DDT-method of Schechter *et al.* (1945) was also satisfactory for the estimation of these chemicals.

The metabolism of Prolan in the resistant strain was studied as follows: Groups of 50 adult female flies, 3 to 4 days old, were anesthetized with CO_2 and the desired dosage of Prolan in 1 microliter of benzene was applied topically to the ventral thoracic region of individual flies by means of an improved ratchet-type microapplicator. The treated flies were held in 600-ml. beakers and were provided with sugar and water. At predetermined intervals ranging from 2 to 120 hours after application, the flies were rinsed with several portions of n-hexane to remove the unabsorbed insecticide, then ground to a fine dry powder with Na_2SO_4 and extracted with ether in a Soxhlet extractor for 8 hours.

Excretory products were collected in a 600 ml. beaker lined with a sprayable strippable film (a product of E. I. DuPont de Nemours & Co., Inc., Wilmington, Delaware) which was peeled off the container at the end of the test and dissolved in methyl ethyl ketone. This solution was transferred to a separatory funnel and shaken with 2% NaOH for several minutes. After separation of the two phases the alkali was decanted, acidified with HCl, and extracted with diethyl ether. By this procedure acidic components resulting from Prolan metabolism were removed successfully from the excreta and any neutral material present remained in the organic solvent. Exploratory tests had ascertained that extraction of the excreta with alkali did not remove unchanged Prolan from the organic solvent phase.

Purification of both acidic and neutral materials was accomplished by evaporating the extracts to near dryness, suspending the residues in CCl_4, and chromatographing through glass columns containing 15 grams of Florisil (a product of Floridin Co., Warren, Pennsylvania), using CCl_4 as eluant. Following evaporation of the eluates the extracts were analyzed by the method of Schechter *et al.* (1945).

For large-scale collection of metabolites, resistant flies in lots of 100 to 150 each were sprayed with 3% Prolan in deobase using a modified Hoskins-Caldwell spray

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chamber (Hoskins & Caldwell 1947). After spraying, the flies were transferred to 4-liter beakers lined with strippable film and were provided with food and water for a period of 48 to 72 hours. At the end of this period the film was removed and dissolved in 30 ml. of methyl ethyl ketone. To this solution was added 60 ml. of acetonitrile. A white precipitate formed which was removed by centrifugation. The supernatant was evaporated, the residue dissolved in CCl_4, and the latter extracted with alkali to remove the acidic component as described earlier. This procedure was followed by chromatography through Florisil. In this manner, milligram quantities of metabolic products were obtained.

Characterization and identification of metabolites was accomplished by:

(1) Colorimetric analysis by the Schechter-Haller method as mentioned earlier;

(2) Colorimetric analysis by the ferric chloride method (Jones & Riddick 1951). This method offers a means of specific characterization for Dilan and its components and is based on the fact that the nitro group attached to a secondary alkane carbon exists in equilibrium with its tautomeric aci-form according to the formula:

$$R_2CHNO_2 \rightleftharpoons R_2C=NO_2H.$$

The equilibrium is shifted to the right in the presence of alkali and the addition of ferric chloride forms a reddish-brown colored complex with maximum absorbance at 490 millimicrons. Absence of the NO_2 group from the molecule yields no color reaction with ferric chloride;

(3) Infrared spectroscopy, using a Beckman IR-3 spectrophotometer with NaCl optics and 0.2 mm. cells; solutions were analyzed in CCl_4 and CS_2. By this method it was possible to show the transformation of Prolan into an acidic derivative, and also the excretion of unchanged prolan;

(4) Ultraviolet absorption spectra, using a Beckman DU spectrophotometer; and

(5) Bioassay of excretory products using 3rd instar *Aedes aegypti* (L.) larvae. Acetone solutions of extracts in 1 ml. aliquots were placed in glass finger bowls containing 20 larvae and 100 ml. of tap water per bowl. The mortalities at 24 and 48 hours were compared with those obtained by exposure of larvae to known concentrations of the pure chemicals which were run concurrently with each unknown sample.

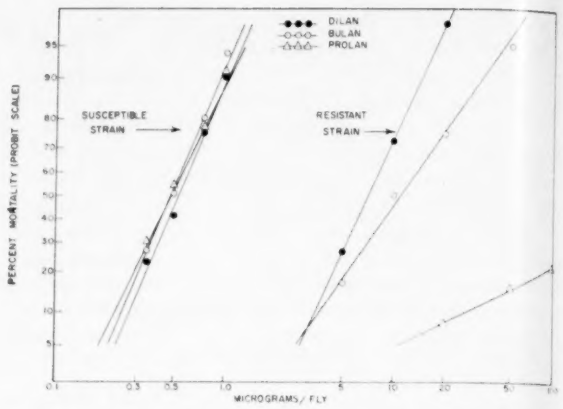


FIG. 1.—Dosage-mortality lines for susceptible (NAIDM strain) and Dilan-resistant female house flies resulting from topical applications of Prolan, Bulan, and Dilan.

RESULTS.—The relative susceptibilities of the resistant and susceptible strains to Prolan, Bulan, and Dilan, by topical application of the chemicals, are shown in figure 1 and table 1. Very little difference was noted in the susceptibility of the normal (NAIDM) strain to Dilan and its components but the resistant strain showed markedly greater resistance to Prolan than to Bulan or Dilan. Although this phenomenon was not investigated fully, it was found, in tests not reported here, that Prolan was excreted more rapidly than either Bulan or Dilan.

The metabolism of Prolan in the resistant strain was followed at intervals ranging from zero to 120 hours. In all cases the zero-hour test was taken as an index of the actual amount of insecticide applied. The averaged results of 4 to 6 tests with each dosage are shown in table 2. These results indicate that Prolan, like DDT, is absorbed at a fairly slow rate. Thus, only 42% of the dosage applied had been absorbed after 24 hours, and as much as 12% was still unabsorbed 5 days after application of the chemical. The amount of unchanged Prolan remaining in the tissues never exceeded 0.5 microgram per fly which is roughly an LD_{50} for the susceptible strain.

By far the largest portion of the metabolized insecticide was found in the excreta. The term excreta is used here to denote fecal material as well as egestion and rub-off.

Table 1.—LD_{50}'s and probit analysis of dosage-mortality data for Dilan, Bulan, and Prolan applied topically to susceptible and Dilan-resistant house flies.

Fly Strain	Compound	LD_{50} μg./Fly	Fiducial Limits P=.05	Chi² Test n	Chi² Test χ^2	Slope ± S.E.	Regression Line Y=
Susceptible (NAIDM)	Dilan	0.53	0.50–0.55	2	0.87	4.55±0.33	1.69+4.55x
	Bulan	0.48	0.45–0.50	2	2.30	4.59±0.35	1.87+4.59x
	Prolan	0.38	0.36–0.41	2	1.30	3.88±0.33	2.72+3.88x
Dilan-Resistant	Dilan	7.0	6.5 – 7.4	1	1.90	3.92±0.27	1.69+3.92x
	Bulan	10.8	9.9 –11.7	2	0.84	2.44±0.14	2.47+2.44x
	Prolan	—[a]	—[a]	1	0.19	0.86±0.28	2.51+0.86x

[a] LD_{50} not reached.

Table 2.—Absorption and metabolism of Prolan by resistant house flies at various intervals following topical application of 8.0 micrograms of Prolan per female fly.

Interval (Hours)	Prolan Recovered (Micrograms/Fly ± S.E.)					Per Cent Recovery
			In Excreta			
	External	Internal	Neutral Material[a]	Acidic Metabolite[a]	Total	
0	8.0 ±0.15	—	—	—	8.0 ±0.15	100
2	7.24±0.18	0.30±0.02	?	?	7.54±0.20	94.2±2.5
4	6.40±0.15	0.44±0.03	0.20±0.03	0.16±0.03	7.20±0.24	90.0±3.0
8	5.80±0.16	0.50±0.04	0.56±0.03	0.40±0.03	7.26±0.26	90.7±3.2
24	4.60±0.24	0.24±0.02	1.45±0.12	1.02±0.11	7.31±0.49	91.4±6.1
48	2.97±0.18	0.23±0.03	1.60±0.17	1.61±0.09	6.41±0.47	80.1±5.8
72	2.00±0.14	0.19±0.02	1.63±0.06	2.19±0.13	6.01±0.35	75.1±4.3
120	0.94±0.08	0.14±0.02	1.95±0.14	3.71±0.16	6.74±0.40	84.2±5.0

[a] Calculated as Prolan.

Mechanical loss to the container due to the flys' activity in a confined space was measured by topical application of C^{14}-DDT. Using unlined beakers for the collection of excretory products, a topical dose of 8 micrograms of C^{14}-DDT per fly yielded in the excreta an average of 1.1 ±0.2 micrograms DDT per fly at the 72-hour interval after application of the chemical. This amount corresponds to 11.2 to 16.2% of the dose applied (cf. Perry *et al.* 1955). A negligible difference was noted in the recovery of DDT or Prolan between the unlined beakers and those lined with strippable film.

Two components were isolated from the excreta; a neutral product and an acidic derivative. Spectral curves of these materials, taken after analysis by the Schechter-Haller method showed that the neutral material with a peak absorption at 545 millimicrons was identical with that of Prolan, whereas the acidic derivative with a peak absorption at 530 millimicrons was more like a breakdown product of DDT. Furthermore, the purplish-color complex of the neutral product was, like Prolan, rather unstable at room temperature, but the pink color of the acidic component was stable for 2 hours or longer. It is also apparent that excretion of both neutral and acidic components continues as long as the insecticide is available but that the ratio of acidic to neutral material increases with increasing time interval. The lower recoveries obtained as the time interval increased may be due to the arbitrary values given for the acidic metabolite. Since the latter has not been positively identified and calibration curves could not be obtained, the assigned values, extrapolated from the Prolan calibration curve, are somewhat arbitrary and might possibly contribute to these lower recoveries.

Characterization of the excretory products of Prolan metabolism was supplemented by the ferric chloride method of analysis as explained under "Methods." Repeated tests showed a positive color reaction for the neutral material and no color for the acidic metabolite. Since this analytical procedure is specific for an aliphatic nitro group it clearly demonstrates the loss of the NO_2 group from the nitropropane moiety.

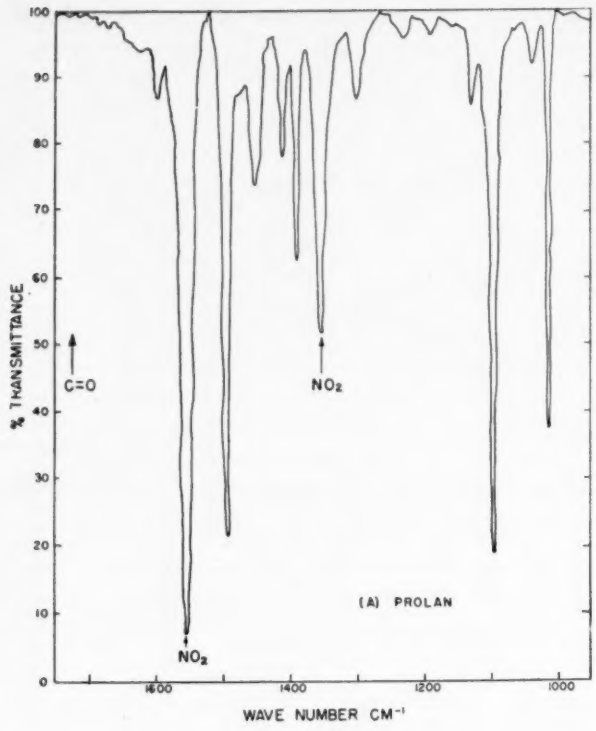
Additional information on the identity of the excretory products was obtained by infrared spectral analyses. A typical infrared spectrogram (fig. 2) of the neutral material showed very close similarity to that of pure Prolan, especially in the regions 1100-1000 cm^{-1} and 1600-1300 cm^{-1}, whereas the acidic metabolite was characterized by a strong absorption peak in the region of 1735-1700 cm^{-1} which indicates the presence of a C=O group, and by the absence of peaks in the regions of NO_2 absorption, again indicating the loss of the nitro group.

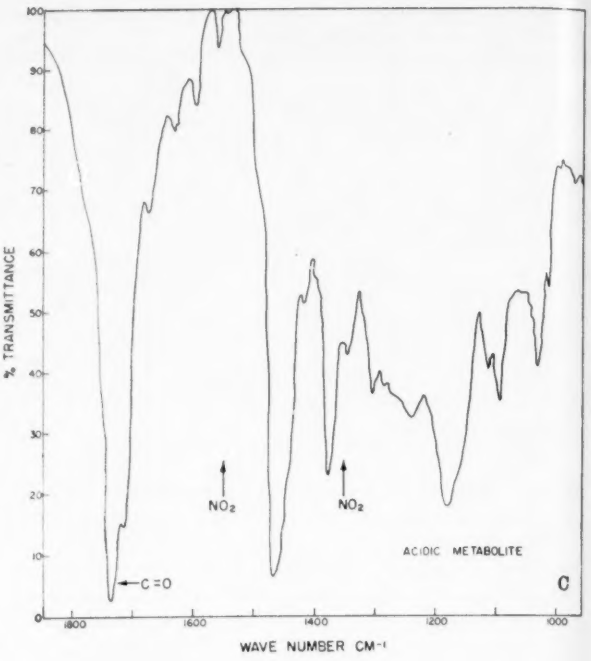
Using a Beckman DU spectrophotometer, ultraviolet spectra of ethanol solutions of Prolan and of the purified neutral excretory product showed identical absorption maxima at 230 millimicrons. The same characteristic curve was obtained with bis-(*p*-chlorophenyl) acetic acid (DDA), which was included for comparison. On the other hand, the acidic metabolite showed a completely different spectrum with a peak absorption at 260 millimicrons, also indicating that the acidic metabolite is not similar to DDA.

Finally, additional substantiating evidence that the excreted material consists of two compounds was obtained by bioassay tests using *Aedes aegypti* larvae. Acetone solutions of the excreted metabolites showed the neutral material was almost as toxic as the parent compound, and the acidic compound was much less toxic (table 3), again indicating the excretion of both un-

Table 3.—Bioassay, using 3rd instar *Aedes aegypti* larvae, of Prolan and the neutral and acidic metabolites recovered from the excreta of Prolan-treated resistant house flies.

Compound	LD_{50} P.P.M.	Fiducial Limits P=.05	Chi² Test		Slope ± S.E.	Probit Regression Line Y=
			n	χ^2		
Prolan	0.072	0.067–0.077	2	0.8	4.37+0.35	1.23+4.37x
Neutral metabolite	0.093	0.086–0.100	2	2.9	3.84±0.31	1.27+3.84x
Acidic metabolite	0.50	0.45 –0.55	2	3.8	2.78±0.23	0.27+2.78x





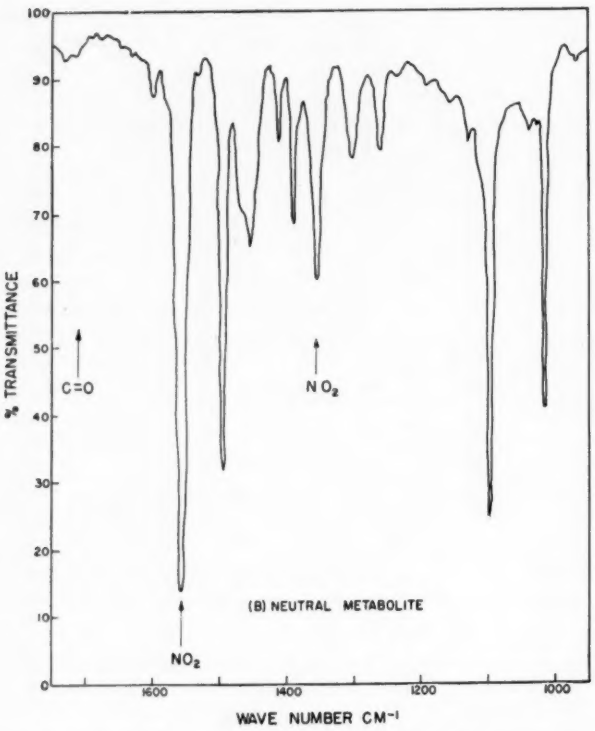


FIG. 2.—Infrared spectra of (A) Prolan and of (B) the neutral and (C) acidic materials recovered from the excreta of Prolan-treated resistant house flies. Beckman IR-3 spectrophotometer, 0.2 mm. cells, NaCl optics. Approximately 3 to 5% solutions in CCl_4, corrected for solvent absorption.

(*See column 2 for Fig. 2-C*)

changed Prolan and of a detoxified product.

The metabolism of Prolan by the susceptible strain was followed in the same manner as that described for the resistant strain. Application of sublethal doses of Prolan (0.2 microgram per fly) yielded 20 to 30% recovery of the applied dose. Only traces of unchanged Prolan were found in the excreta. On the other hand, 77 to 92% recovery was obtained from application of a lethal dose of 4 micrograms of Prolan per fly (table 4). Only small amounts of Prolan were detected in the excreta.

Essentially, the same recovery pattern was obtained when 0.2 microgram Prolan per fly was applied to the resistant strain. However, as shown in table 2, application of larger dosages resulted in the excretion of substantial quantities of the two previously described metabolites.

It is often of interest to show the cross-resistance pattern of a selected strain to other related and unrelated insecticides. The cross-resistance pattern of the Dilan strain is shown in figure 3. In addition to having varying degrees of resistance to all the chlorinated insecticides tested, this strain also exhibited a measurable level of tolerance to organophosphorous compounds such as malathion and Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate).

DISCUSSION.—The results of this investigation demonstrate for the first time the excretion of an insecticide in the unchanged state as at least a partial means of protection against the lethal action of the chemical. Likewise, it is the first definite isolation from fly excreta of an acidic metabolite resulting from the metabolism of one of the newer hydrocarbon insecticides.

Although the acidic metabolite has not been completely identified, it has been established that it contains no aliphatic nitro group, that it manifests a strong infrared absorption peak at a C=O region, that its color and stability in the Schechter-Haller procedure are different

Table 4.—Absorption and metabolism of sublethal and lethal doses of Prolan by susceptible house flies (NAIDM) 24 hours after topical application of Prolan.

Dose μG./Fly	Prolan Recovered (μG./Fly ± S.E.)				Per Cent	
	External	Internal	Excreta	Total	Recovery	Mortality
0.2	0.0	0.05±0.01	0.0	0.05±0.01	25.0±5.0	3–5
4.0	1.0±0.10	2.25±0.20	0.15±0.01	3.40±0.31	85.0±7.7	100

from the parent compound, that it is removed from solution by dilute alkali, and that it is considerably less toxic than Prolan to mosquito larvae. In addition, it has been shown by ultraviolet spectral analysis that it has different absorption characteristics from the parent compound and from the neutral material found in the excreta, and also that it differs from DDA in this respect. It is conceivable that the end product might be 3,3-bis-(*p*-chlorophenyl) pyruvic acid (Hass 1958). However, final conclusions as to the identity of the metabolite must await the synthesis and characterization of the latter compound or similar acidic derivatives of Prolan.

With regard to Prolan metabolism by the susceptible strain it appears that sublethal doses are largely detoxified to unidentified products since in no case was total recovery quantitative. The same applies to the resistant strain at similar dosage levels but at higher dosages excretion of the insecticide and its breakdown product assumes a predominant role.

In elucidating the protective mechanism involved in Prolan resistance it appears that excretion of the acidic metabolite is not of primary importance to the fly's survival since it is produced in substantial quantities only after the period of greatest danger to the fly has passed, *i.e.*, between 12 and 24 hours after application of the toxicant. In this respect excretion of the toxic material might be of greater importance in the over-all protective mechanism.

Although it is implied here that resistance is partly due to excretion of the chemical it may be argued that metabolism of Prolan at high dosage levels is the result rather than the cause of resistance due to the fly's ability to survive longer in the presence of the toxicant. Indeed, it is highly important to demonstrate whether detoxication of the insecticide in one form or another is the cause or the effect of resistance, but this matter cannot be decided solely by experiments *in vivo*. If we assume that such reactions are enzymatically controlled, it is necessary to demonstrate the quantitative aspects of metabolism *in vitro*.

On the other hand, excretion of a toxicant or its breakdown products in relation to resistance is more difficult to evaluate. What governs the rate of excretion of a chemical in an insect? If resistance is due to more rapid excretion of the chemical, what causes this accelerated rate? Evidently, this process is not altogether enzymatically controlled in the case of Prolan, since part of the chemical is excreted unchanged, but there exists the possibility that the toxicant might be excreted in a loosely conjugated form which is easily extractable with organic solvents. Conjugation processes have not been stressed before in relation to insecticide metabolism and resistance, but a review by Smith (1955) indicated that various injected chemicals are excreted by many insect species as glucoside or ethereal sulfate conjugates. Similarly, Perry *et al.* (1958) have suggested that conjugated metabolites might be present in fly excreta as end products of heptachlor metabolism.

Whether the protective mechanism which allows resistant flies to survive the lethal effect of toxicants is the direct result of metabolism of the compound or its excretion, it is an inescapable fact that the toxicant must, in some way, be prevented from reaching the site of action or be removed from it. Our previous knowledge indicated that this can be accomplished by (1) detoxication and retention of harmless metabolites, (2) detoxication and excretion of nontoxic metabolites, and/or (3) storage of toxic products or unchanged material in nonsensitive tissues. Resistance to Prolan adds another facet to this already complicated problem; *i.e.*, (4) excretion of the unchanged insecticide.

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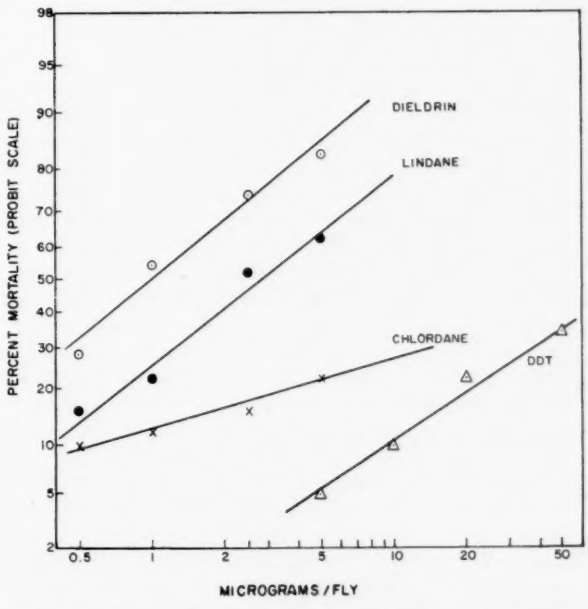

Fig. 3.—Cross resistance of the Dilan-resistant strain to other chlorinated hydrocarbon insecticides. The LD_{50}'s for the susceptible (NAIDM) strain were, in each case, less than 0.4 micrograms per fly. Each point on the curve is an average of three replications using 50 flies per replication.

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Bee Visitors to Saguaro Flowers[1]

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ABSTRACT

Honey bees (*Apis mellifera* L.) begin visiting flowers of the saguaro, or giant cactus (*Carnegiea gigantea* (Engelm.) Britt. & Rose), at dawn, collecting pollen for a couple of hours and nectar until shortly after noon. A single flower may produce 3 ml. or more of nectar, containing about 25% of sugar, and a dozen or more bee loads of pollen. Honey bees are the predominant visitors although other bees also visit the flowers. The latter visit flowers on different trees freely. Honey bees tend to confine their visits to individual flowers or branches (arms) of one plant when nectar is abundant, but visit more widely scattered flowers when competition is strong. The effectiveness of honey bees in pollination of saguaro appears to depend on their population in proportion to the number of available flowers.

The saguaro, or giant cactus (*Carnegiea gigantea* (Engelm.) Britt. & Rose), is seldom found in concentrated stands. Three concentrated areas occur, each covering several square miles, near Tucson, Arizona. They are the western slopes of the Tucson Mountains, the southern slopes of the Santa Catalina Mountains, and in the Saguaro National Monument on the western slopes of the Rincon Mountains. Beekeepers near these areas state that the saguaro produces a mild-flavored, light-colored honey of a high moisture content. They also consider it a good source of pollen. Pellett (1930) lists it as "a source of surplus honey little known in the United States outside of Arizona."

Further interest in the activity of the honey bee (*Apis mellifera* L.) and other pollinating insects on the saguaro was stimulated when it was found that cross-pollination is essential to its fruiting (Alcorn *et al.* 1959). The present paper concerns observations made in connection with this work to determine (1) the value of the saguaro to honey bees as a source of food and (2) the value of honey bees to the saguaro as pollinators. Most of these observations were made in the Saguaro National Monument[4], and most of the bees probably came from an apiary of about 30 colonies one-half mile west of the Monument border, a mile from the site of observations.

FLOWERING HABITS OF SAGUARO.—The flowering period of saguaros extends from about mid-May to mid-June. The individual flower opens between 9 P.M. and midnight (Kearney & Peebles 1942) and remains open until the following afternoon. It is usually completely closed by 5 P.M. and does not open again.

Pollen is released on the numerous anthers during the night as the flower opens, and honey bees begin collecting it at dawn. In doing so they hover over the anthers, dipping down frequently to fan or rub pollen onto their body hairs; then, while still hovering, they comb it into the pollen baskets, packing it into cream-colored pellets, on the hind pair of legs. Several honey bees were seen collecting their entire load of pollen from a single flower. Continuous bee visits to one flower indicated that it produced a dozen or more such bee loads. The large number of flowers available to honey bees in a saguaro forest establishes this plant as a major pollen source.

Nectar is secreted from a ring around the base of the style. Its aroma is mildly pleasant, similar to that of under-ripe cantaloup. The sugar concentration of the nectar is about 25%, similar to nectar of Pima cotton (*Gossypium barbadense* L.) but much less than that of such common plants as alfalfa, apple, or sweetclover (Vansell 1942).

The nectar-collecting bee alights on the flower and immediately burrows out of sight below the anthers to reach the nectar. In so doing it becomes coated with pollen. When it crawls out of the flower it attempts to comb this pollen off its body, but makes no attempt to pack it in its pollen baskets. No honey bee was seen collecting both pollen and nectar on a single trip.

Two or three ml. of nectar was collected by pipette at one time from individual flowers caged to exclude bees and other nectar-collecting insects. Total nectar production in a flower was estimated to be about 5 ml. When such caged flowers were turned downward, several drops of nectar would fall from each. This amount far exceeds that produced in most other flowers. Flowers of Pima cotton, for example, yield only about 0.1 ml. Upland cotton flowers (*G. hirsutum* L.) yield less. Fahn (1949) ex-

[1] Arizona Agricultural Experiment Station Technical Paper 516. Accepted for publication May 4, 1959.

[2] Respectively Entomology Research Division and Crops Research Division, Agric. Res. Serv., U.S.D.A., Tucson, Arizona.

[3] College of Agriculture, University of Arizona, Tucson.

[4] Appreciation is expressed to Saguaro National Monument personnel for their cooperation in these studies.

amined 66 species of wild and cultivated plants and found that nectar production ranged from 0.13 mg. in *Thymus capitatus* (Hoffmgg. & Link) to 268 mg. in *Musa paradisiaca* var. *sapientum* L. (roughly from 0.0001 to 0.3 ml.).

Within the main saguaro areas there are about 16 trees per acre, each bearing an average of four flowers per day over a 30-day period. The secretion of 5 ml. of nectar per flower would amount to 320 ml. per acre, about half a pound per day. This is similar to the calculated production of nectar of cantaloups (0.3–1.7 pounds per acre) but far less than alfalfa (54 to 238 pounds per acre) (McGregor & Todd 1952). Obviously there would be more than the average number of flowers during the peak of saguaro flowering. Because of the extensive acreage of saguaros in the Tucson areas as compared with the size of the usual melon or alfalfa fields, the amount of nectar produced during the flowering period, about one-half ton per day within a mile radius, should enable a 50-colony apiary in such a location to store a surplus of honey.

BEE VISITORS.—To obtain definite information on visits to saguaro flowers by honey bees and other pollinating insects, a circular crown of seven open flowers on the tip of a branch (arm) of a plant was observed continuously from 5 A.M. to 12:45 P.M. on May 21, 1958. These seven flowers were visited 400 times by honey bees and eight times by unidentified species of wild bees, or about one visit to a flower every 8 minutes. Of the honey bee visits 60 were by pollen collectors and 340 by nectar collectors. Figure 1 shows the relative abundance of these visitors at different times in the day. Pollen collection ceased about 2 hours after dawn, but nectar collection continued until shortly after noon, when the flowers began to wither.

On June 5, during a 50-minute period, 112 honey bees and 30 wild bees visited 7 flowers on an arm, 1 visitor per flower every 148 seconds. Eight days later an attempt was made to count bee visitors to four flowers on an arm. During the early part of the morning the flowers were being visited about every 20 seconds, too frequently for accurate counts to be made. Visitation continued at a high rate even after cessation of pollen collection. Visits by individual bees to several blossoms indicated that the nectar was being removed as fast as it was secreted, so the bees could not get full loads from individual flowers.

VISITS BY AN INDIVIDUAL HONEY BEE.—In the course of these observations a particular honey bee, recognizable from the others by its black color, was first observed on a certain flower (No. III in fig. 2) preparing for departure to its domicile with a load of nectar. (This domicile was never located but must have been nearby in the forest, as the homeward flight by the laden bee was not toward the known apiary.) This bee departed about 9:10 a.m. and returned to the same flower at 9:17 a.m. It obtained a load of nectar from this flower and departed at 9:24 a.m. It was recognized many times thereafter until 12:28 p.m., and its time of arrival and the flowers visited recorded (fig. 2)—a marvel in the constancy of the honey bee in foraging the same limited area. On several trips it visited only one flower, and it showed no inclination to visit flowers on other arms of this plant or other saguaros.

WILD BEE VISITORS.—Other identified species of bees collected on saguaro flowers during the period of observation were as follows:[5]

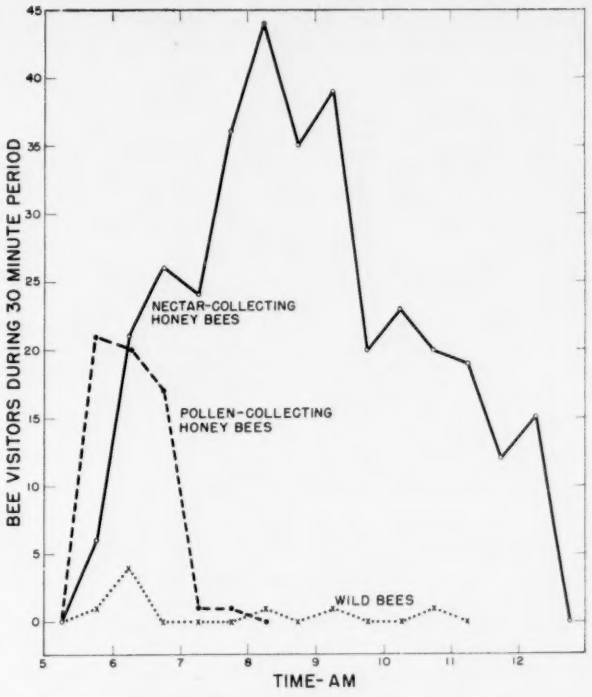

FIG. 1.—Bee visitors to seven saguaro flowers between 5 A.M. and 12:45 P.M. on May 21, 1958. Saguaro National Monument.

Bombus sonorus Say
Didasia opuntiae Ckll.
D. rinconis Ckll.
D. sp.
Melissodes sp.
Agapostemon melliventris Cress.
Augochlorella pomoniella (Ckll.)
Halictus (*Chloralictus*) sp.
Perdita spp.

None of these bees were common throughout the flowering period, nor were their combined numbers at any time comparable to those of the honey bee visitors (fig. 1). The wild bees in general visited flowers on different plants more freely than honey bees, although many of the males did not contribute to cross-pollination. They entered flowers only infrequently to feed on nectar or to mate. The females collected nectar and pollen on the same visit, and where sufficiently abundant could be of major importance in the transfer of pollen between flowers.

DISCUSSION.—In the study of the pollination requirements of the saguaro (Alcorn *et al.* 1959) one treatment consisted of arms wrapped with window screens so that about a foot of the screen extended, tubelike, beyond the end of the arm. A cage was formed over the flowers by covering the open end of this tube with cheesecloth. About 50 live honey bees were released into each of five such cages when the first flowers opened, and this population was maintained throughout flowering by the addition of bees when necessary.

These bees, although deprived of their normal domicile

[5] Appreciation is expressed to K. V. Krombein of the Entomology Research Division, U. S. Department of Agriculture, for the insect identification.

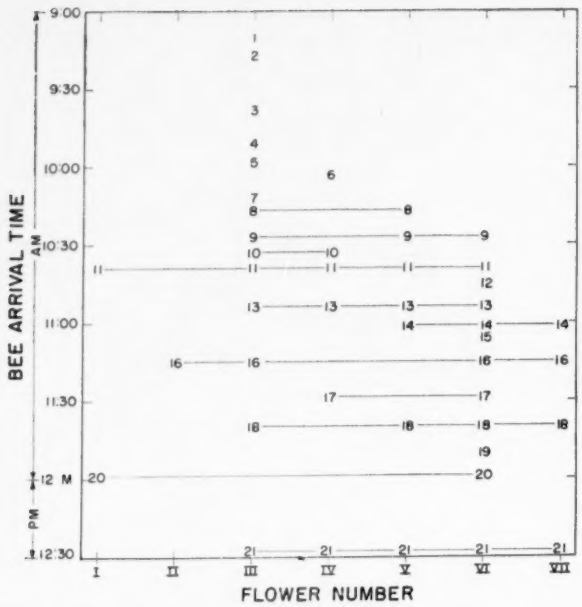

FIG. 2.—Visits by a single honey bee to seven flowers on a saguaro arm between 9 A.M. and 12:30 P.M., May 21, 1958. The numbers indicate the trip numbers and the lines the visits to different flowers on a single trip. Numbers without lines indicate that a complete load of nectar was obtained from the one flower. The bee returned to its domicile after obtaining each load, making 21 known trips.

and activities, fed freely on the floral nectar. The failure of fruit to set in these cages reflects the self-sterility of the flowers, although there may have been lack of pollen transfer from anthers to stigma between the flowers by the bees. Further evidence of self-sterility was the failure of fruit to set in cages where bees were excluded and the flowers were hand-pollinated with pollen from the same blossom. A high percentage (80%) of fruit set when stigmas received pollen from flowers of other saguaros (Alcorn *et al.* 1959).

In view of these results the observations on the repeated visits by one honey bee to a single flower or to flowers on a single arm would indicate that honey bees were of no value as pollinators of saguaro. This could be true if flowers received only occasional visits because of a low bee population, or when individual plants within an area varied greatly in their attractiveness to bees. At the time this particular bee was observed (May 21) palo verde trees (*Cercidium microphyllum* (Torr.) Rose & Johnston) were in full bloom and attractive to honey bees. When observations were made on June 13 palo verde had ceased flowering and honey bees were competing with each other for the saguaro nectar and were thus forced to visit many flowers to obtain their load of food. Under such circumstances they could be effective cross-pollinators of saguaro. Similar observations have been made on visitation of honey bees in the cross-pollination of cotton. When the floral visitor population on cotton is low, a bee can obtain a load of nectar from a single flower, without effecting cross-pollination. When the population is high, so that nectar is being removed as fast as it is secreted, a bee has to enter many flowers to obtain a load of nectar, and thus is more likely to effect cross-pollination.

The effectiveness of honey bees as pollinators of saguaro therefore depends on the population in proportion to attractiveness of the saguaro and other plants.

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Tests with Dimethoate for Systemic Control of Cattle Grubs[1]

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ABSTRACT

Cattle from Kerrville and College Station, Texas, and Highmore, South Dakota, were treated orally, intramuscularly, or dermally with dimethoate. Oral and intramuscular dosages ranged from 5 to 20 mg./kg. and the strength of dermal sprays from 0.25 to 1%. Cattle grub control ranged from 10% to 96%, depending on the dosage. Those animals treated at the higher dosages were poisoned by the insecticide. There does not appear to be an adequate margin between the dosage that is effective against first-instar *Hypoderma* larvae and the dosage toxic to cattle.

Dimethoate, also known as Am. Cyanamid 12880, has shown considerable promise as an animal systemic insecticide in screening tests at the Kerrville, Texas, laboratory. It has killed screw-worms (*Callitroga hominivorax* (Cqrl.)), stable flies (*Stomoxys calcitrans* (L.)), and lone star ticks (*Amblyomma americanum* (L.)) feeding on guinea pigs and goats at dosages that were not apparently toxic to the mammalian hosts (Drummond 1958). The compound showed systemic activity in screening tests in which yellow-fever mosquitoes (*Aedes aegypti* (L.)) were allowed to feed on treated mice, and in supplementary tests with other mammals (Hewitt *et al.* 1958). Also, Hewitt *et al.* (1958) have reported that, in preliminary tests, this compound killed all three larval stages of the

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common cattle grub (*Hypoderma lineatum* (De Vill.)) and the northern cattle grub (*H. bovis* (L.)) when administered both orally and intramuscularly to cattle.

In 1957 tests were conducted with cattle from ranches near Kerrville and College Station, Texas, and Highmore, South Dakota, to determine the dosages of dimethoate necessary to control cattle grubs.

TESTS WITH CATTLE FROM KERRVILLE, TEXAS.—Yearling steers and heifers from two herds of Hereford cattle were treated orally, intramuscularly, and dermally with dimethoate. The cattle were treated less than a month before grubs were due to reach their backs. For the oral and intramuscular treatments a stock solution was prepared by combining equal portions of dimethoate and absolute alcohol. This solution was diluted with distilled water to obtain a solution containing 20% of the active ingredient. For dermal treatments an emulsifiable concentrate was prepared by dissolving 25 parts of dimethoate in a mixture of 65 parts of xylene and 10 parts of Triton X-100, and water was added to make different concentrations.

Thirty animals from herd 1 were divided into four groups, and those in each group were numbered with a special marking dye. Fourteen animals were weighed and given the insecticide orally on July 26. The stock solution was mixed with water to a volume of approximately 4 ounces and administered with a 4-ounce drenching gun. Seven cattle were sprayed with a 0.5% emulsion of the insecticide applied at the rate of 1 gallon per head with a power sprayer operating at a pressure of 100 pounds per square inch. The remaining nine cattle, not treated, served as controls.

From herd 2 fourteen heifers were purchased and moved to the Kerrville laboratory. Each animal was numbered with a metal ear tag and weighed. On July 23 an appropriate amount of the stock solution was injected into muscle of the gluteal region of eight animals. Six animals were not treated.

Animals from herd 1 were examined for grubs at monthly intervals from August through December, and those from herd 2 from August through January 1958. Outline maps were made of the backs of the animals. As grubs encysted, they were located on these maps so that cumulative grub counts could be made. A t test was used to determine the significance of the differences in average number of grubs per group.

The results of the tests with cattle from Kerrville are summarized in table 1. In herd 1, 10 mg./kg. gave a reduction in numbers of grubs significant at the 0.01% level. The other two treatments afforded some grub control, but the reductions were not significant at the 0.05% level. In herd 2, neither treatment gave a significant reduction in number of grubs.

TESTS WITH CATTLE FROM COLLEGE STATION, TEXAS.—The American Cyanamid Company purchased a group of yearling Hereford steers and heifers and moved them from College Station, Texas, to Greely, Colorado. Twenty of these animals were then shipped to pastures at Camp Stanley, Texas, for treatment by the author, and the others remained at Greely. Each animal was marked with a firebrand and a tattoo.

Sixteen animals were sprayed with an emulsion of dimethoate. Each animal received 1 gallon from a power sprayer operating at a pressure of 100 pounds per square inch. Cattle were treated more than a month before grubs appeared in the animals' backs.

The animals were checked for encystment of grubs at monthly intervals from September through February, and cumulative counts of grubs for each animal were maintained.

Results of this test are summarized in table 1. Grubs failed to appear in one or more animals in each group. Fewer grubs were found in cattle sprayed with both concentrations of dimethoate than in the untreated animals, but the differences were not significant.

TESTS WITH CATTLE FROM HIGHMORE, SOUTH DAKOTA.—Hereford cattle 8 to 9 months old were obtained from a ranch near Highmore, South Dakota, where *Hypoderma lineatum* and *bovis* were known to occur (McGregor & Bushland 1957, Graham 1958). In October 1957 they were moved to Kerrville.

Twenty-eight animals were treated with dimethoate orally as a drench, intramuscularly, or dermally. The drench was prepared from a 25% emulsifiable concentrate. The injectable material was furnished by Lederle Laboratories. Calves, in groups of four, were weighed and treated orally or intramuscularly on November 12. On

Table. 1.—Grubs from cattle treated with dimethoate, 1957.

Treatment	Dosage[a]	Number of Animals		Average No. of Grubs per Animal	Per Cent Control
		In Test	Infested		
	Kerrville Herd 1 (Treated July 26)				
Drench	10	7	7	8.4	65
	5	7	7	13.1	45
Spray	0.5%	7	5	12.6	48
Untreated	—	9	9	24.1	
	Kerrville Herd 2 (Treated July 23)				
Injection	10	4	4	16.5	51
	5	4	4	29.2	13
Untreated	—	6	6	33.5	
	College Station Herd (Treated August 12)				
Spray	1%	8	6	13.0	60
	0.25%	8	7	18.9	37
Untreated	—	24[b]	20	30.2	
	South Dakota Herd[c] (Treated November 12–14)				
Drench	10	4	4	31.25	14
	15	4	3	7.0	81
	20	4	4	2.5	93
Injection	10	4	4	3.0	92
	15	4	3	7.2	80
	20	4	4	2.7	93
Spray	0.75%	4	3	1.5	96
Untreated	—	20	20	36.3	

[a] For drenches and injections, milligrams of dimethoate per kilogram; for sprays 1 gallon of emulsion per head for Kerrville and College Station herds, 1½ gallons for South Dakota herd.

[b] Data from 20 animals furnished by J. J. Drane, American Cyanamid Co., Greeley, Colorado.

[c] Both *Hypoderma bovis* and *H. lineatum*.

November 14 four animals were sprayed with a 0.75% emulsion applied at the rate of 1½ gallons per head with a power sprayer operating at a pressure of approximately 100 pounds per square inch. Twenty animals were not treated. The calves were checked for encystment of grubs five times from January through April 1958.

The results of these tests are summarized in table 1. None of the treatments gave complete control of grubs. Grubs failed to appear in one animal in each of three treatment groups. The control varied directly with the dosage, except that the injection of 10 mg./kg. was better than 15 mg./kg. The spray treatment, all the intramuscular treatments, and all except the 10 mg./kg. oral treatment gave highly significant reductions in number of grubs.

DISCUSSION.—There was considerable variation in the results of treatments at 10 mg./kg., control ranging from 14 to 92%. When higher dosages were used, this variation was not evident. At the dosages used, there was no consistent difference based on method of administration. Radiometric studies with dimethoate (Kaplanis *et al.* 1959) have shown that radioactive compounds remain in the blood longer when administered orally, but they reach higher peaks when administered intramuscularly. Data from the tests reported herein indicate these differences are not reflected in control of grubs.

In general, control afforded by spray treatments increased with the concentration of dimethoate; however, the 0.75% spray gave the best results. The animals sprayed with 0.25, 0.5, and 1% concentrations were all in short summer hair at time of spraying, and some of the insecticide ran off their bodies. Each animal received about 1 gallon. With the 0.75% spray each animal was treated with 1½ gallons. Because these animals were brought from South Dakota in the late fall, they had long, heavy coats of hair. Although some of the insecticide did run off, it is believed that larger amounts were retained on the wet hair in contact with the bodies, and possibly more was absorbed into the tissues.

None of the animals from the two herds near Kerrville appeared to be poisoned by the insecticide. Some of the animals from College Station were noticed by the herdsmen to act unnaturally after spraying. Cholinesterase analyses of posttreatment blood samples[2] indicated that the animals treated with 1% spray had been mildly poisoned.

South Dakota cattle treated at 20 mg./kg. orally and intramuscularly and at 0.75% dermally were examined closely for symptoms of poisoning. The animals treated orally were moderately or severely poisoned, and two treated intramuscularly were moderately poisoned. All animals, including those sprayed, showed considerable reduction in blood cholinesterase activity. All the cattle recovered without any apparent aftereffects.

These results indicate that dimethoate is not toxic to cattle at low dosages, but the dosages required to control grubs are only slightly below the toxic level.

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Culture Methods for Mass Rearing of Screw-worm Larvae[1]

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ABSTRACT

Screw-worm (*Callitroga hominivorax* (Cqrl.)) larvae were reared in sufficient numbers to produce 2 million sterilized flies weekly for 15 consecutive weeks in a Florida field test. Five days each week eggs were obtained from 20 colony cages, each stocked with 700 eight-day-old flies, by heating an attractive medium in the cages. Single cultures of 100,000 larvae were reared on a mixture of ground lean meat, blood, and water, in vats. Until irradiation as 6-day-old pupae, the insects were held at 80° F. and 90+ per cent relative humidity.

The purpose of this research was to develop methods for consistently rearing millions of screw-worm flies (*Callitroga hominivorax* (Cqrl.)) for sterilization and field releases in Florida. Laboratory experiments (Bushland & Hopkins 1951, 1953) at Kerrville, Texas, showed that screw-worm flies could be made sexually sterile by irradiation in the pupal stage with X-rays and gamma rays. In a field test (Baumhover *et al.* 1955) the release of 400 sterile males per square mile weekly for 5 months eradicated this insect from the 170-square-mile island of Curaçao, Netherlands Antilles.

In nature screw-worms develop only in the living tissue of warm-blooded animals, causing serious injury or death. Although this is an obligatory parasite, larvae were reared successfully for many years on artificial media

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(Melvin & Bushland 1941) consisting of mixtures of lean ground meat, blood, and water. Eggs were obtained by holding several females in shell vials with a little lean meat for about 2 hours at 95° F., and cultures of larvae were grown in washtubs or bun trays in a room or cabinet heated to about 100° F. This method was well adapted to rearing thousands of insects, but not for producing millions. Procedures by which 2 million screw-worms were reared per week for 15 consecutive weeks in a pilot plant near Bithlo, Florida, are presented in this paper.

ADULT FLY COLONY.—The laboratory colony was the progeny of a composite strain of flies taken from several localities in Florida and adapted to a laboratory environment by Baumhover and New (unpublished).

In mass rearing, 3% of the total flies produced were used as breeding stock for the propagation of new cultures. Breeding stock in sufficient numbers to produce 2 million flies per week was held at 80° F. in disposable cardboard cages 22×12×12 inches with sleeves on one end. Since eggs were needed for new cultures on a 5-day-per-week schedule, the colony-holding cages were stocked accordingly. Each day 25 cages were stocked with 700 flies of both sexes in the last day of the pupal stage. A meat-honey mixture and a honey fountain were inserted just prior to adult emergence and no other feeding was required. Water was provided by inserting a pint jar over paper toweling in a petri dish.

OVIPOSITION.—Oviposition of 200 to 300 eggs per female was obtained from 8-day-old flies held at 80° F. Because of high mortality of older flies, eggs were obtained only once.

For large-scale rearing, gravid females were stimulated to oviposit on a special medium in the colony holding cages. The attractive ingredient was the fluid recovered from the thawing of frozen ground beef heart and incubated for 72 hours at 97° F. One part of the fluid was thoroughly mixed with nine parts of lean ground meat. To obtain eggs, two wide-mouth pint jars, each containing 50 grams of the medium, were placed in each cage. Cardboard cylinders held the jars above 15-watt light bulbs, maintaining a constant temperature of 97° F. at the surface of the medium. After 2 hours oviposition was completed and eggs were collected and weighed into 10-gram lots, each lot consisting of approximately 200,000 eggs. More than 99% of the eggs hatched on moistened paper in petri dishes after 14 to 16 hours' incubation at 80° F.

Rearing 2 million screw-worms per week required 200 grams of eggs, which were obtained from flies in approximately 100 colony cages. For future production of 50 million flies per week a colony cage with 60 times the fly-holding capacity was developed. This was a wood-supported screen cage 6×6×4 feet. One entire end was a door for entering and servicing. Resting area for 50,000 flies was provided by suspending paper toweling from a rack near the top of the cage. Containers with food and water were placed on the floor along the sides, and the center left free for an electrically heated oviposition tray 5 feet long, 10 inches wide, and 1 inch deep. To obtain eggs the tray, with oviposition medium ½ inch deep, was inserted through a sleeve in one end of the cage. By this method on an average 135 grams (2,700,000 eggs) were collected from each cage.

REARING OF LARVAE.—Equipment and procedures were developed for weekly rearing of 20 cultures, each of 100,000 larvae. Larvae were grown on a medium containing 54% lean ground meat, 30% water, 15% citrated bovine blood, and 0.2% formalin to retard putrefaction. Satisfactory growth was obtained on horse meat, beef, or beef heart not exceeding 9% in fat. The medium was mixed with a double propeller agitator in a 30-gallon soup kettle and pumped directly through a hose to the rearing facilities. Each culture required approximately 150 pounds of meat, 5 gallons of blood, and 10 gallons of water.

Larvae were grown the first day in galvanized-iron starting trays 18×24 inches and 4 inches deep. Since newly hatched larvae are only surface feeders, the surface of the medium was kept warm by holding the trays in a constant-temperature cabinet at 95° F. and 100% relative humidity. Growth was completed in galvanized-iron vats 10 feet long, 4 feet wide, and 1 inch deep. Each vat formed the top of a table with the bottom and sides enclosed. Galvanized-iron troughs, fitted to all sides, caught and directed the full-grown larvae to sand tubs on the floor. For convenient servicing the vats were spaced 2½ feet apart in rows. Instead of being held in a heated room, as had been the practice, the rearing medium was heated from beneath with a battery of light bulbs, and larvae were reared at temperatures closer to those of nature. Although the uninfested portions of the medium were heated to 95°, the larvae fed as a colony and within the mass of feeding maggots the temperature was 5° to 7° higher. The surrounding air of the well-ventilated room was much cooler. Constant air circulation also prevented the accumulation of ammonia gas and disagreeable odors from the products of larval metabolism and putrefaction of the medium.

With the equipment and conditions described above, larvae were fed once daily a quantity of medium for their age requirements, and they attained full growth after 5 or 6 days. Four new cultures were started simultaneously on five consecutive days per week. Each culture of newly hatched larvae spent the first day on medium ½ inch deep in the starting trays. Their growth was accelerated by substituting blood serum, when available, for whole blood in the starting medium. On the second day each culture was transferred to a rearing vat. However, the vat was not filled with medium at this time, but the larvae were established in strips of medium ¾ of an inch deep, 10 inches wide, and running the full length of the vat. The medium was held within this area by attaching a thin sheet of plastic to a board which formed a temporary division within the vats. On the third day this board was removed and medium ¾ inch deep was pumped in, completely filling the vat. Screw-worm larvae feed gregariously, migrating in a path across the vats, leaving behind the spent medium. All spent medium was scooped out with dustpans before the fourth day's feeding, and the larvae mixed with the remaining food were arranged in a central area 1 foot wide and 10 feet long. Medium 1 inch deep was pumped into the space on both sides to allow feeding in opposite directions from the center. Procedures on the fifth, and final, day of feeding were similar, except that less medium was added because most of the larvae had attained full growth. At this time enough

spent medium was removed from the central area to make room for a layer of medium 1 inch deep, 10 inches wide, and lengthwise bordering the collecting troughs. Grown larvae, seeking soil in which to pupate, left the vats and were collected as described above. After collection of each culture, the vats were washed and sterilized with steam. All spent medium was steamed to kill any remaining larvae, and then covered with dirt in trenches.

PUPATION.—Grown larvae were transferred from the collecting tubs beneath the vats to sand in pupation trays 24×24×5 inches. To prevent overcrowding, not more than 10,000 larvae were placed in 4 liters of sand in each tray. New pupae were separated from sand and larvae every 12 hours. Sand was removed by sifting through a screen-bottom tray. Separation of larvae from pupae was accomplished by holding a mixture of both above pupation trays in wire baskets of $\frac{1}{8}$-inch mesh, through which the larvae crawled and dropped to the sand below, leaving the pupae behind.

HANDLING OF PUPAE.—Newly formed pupae were distributed at the rate of 2 liters to each wire holding tray of 18×26×2 inches, washed with tap water, and held at 80° F. and 95% relative humidity. After $5\frac{1}{2}$ days 97% of the pupae were delivered to the sterilization facilities, the other 3% being diverted to colony maintenance.

RECORDS ON PRODUCTION.—Following the procedures described above, screw-worms were reared from April 15 to August 11, 1957. A crew of men (one entomologist, one biological aid, and laborers) reared 37,458,000 pupae in a total of 4,231 man-hours. The insects consumed 106,600 pounds of rearing medium.

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Systemic Insecticides for Citrus Whitefly Control on Gardenia[1]

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ABSTRACT

Experiments were conducted at the Ornamental Horticultural Field Station, Mobile, Alabama, for 4 years beginning in 1953 on control of citrus whitefly, *Dialeurodes citri* (Ashm.), on 1-year old liners of *Gardenia jasminoides* v. *radicans* and v. *mystery*. Treatments were replicated four times and randomized. Demeton 50%, methyl demeton 50%, Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl) phosphorodithioate) 48%, Am. Cyanamid 12009 (*O,O*-diethyl *S*-(propylthio)methyl phosphorodithioate) 48%, and Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) 25%, all at the rate of 1 pint per 100 gallons of water, and white oil emulsion at 2 gallons per 100 gallons of water were highly effective for control of whitefly. Schradan, malathion, Parathion, and Isolan® (dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate) foliage sprays were less effective against this insect. Soil applications of demeton, Am. Cyanamid 12008, Am. Cyanamid 12009, Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)-ethyl phosphorodithioate) were also highly effective for whitefly control. Two foliage or soil applications were necessary for best control.

The citrus whitefly, *Dialeurodes citri* (Ashm.), feeds on a wide variety of plants. Its hosts include 15 primary and 16 secondary plant genera and species (Armitage 1954). Damage to citrus by the citrus whitefly has been recognized for many years (Watson & Berger 1937, English & Turnipseed 1940). In Alabama, gardenia is a favorite host plant of this insect. The whitefly is active throughout the year with peak populations occurring during late summer and fall. Feeding is confined almost entirely to the underside of leaves. Leaves become black from the growth of a sooty mold in the excrement of the immature stages. Heavy infestations lower plant vitality and flower and fruit production. Infested plants are also more susceptible to cold injury than healthy plants (English & Turnipseed 1940, Tippins 1958).

White oil emulsion was the standard recommendation for citrus whitefly control in Alabama for many years (English & Turnipseed 1940). Oil sprays were applied during periods of relatively mild weather to avoid injury to plants. The effectiveness of several systemic insecticides for citrus whitefly control on gardenia even during periods of subfreezing temperatures was reported by Tippins (1958).

Experiments were conducted at the Ornamental Horticultural Field Station, Mobile, Alabama, for 4 years beginning in 1953 on control of the citrus whitefly on two varieties of *Gardenia jasminoides*. Several organophosphates were applied to the soil or foliage and compared with the effectiveness of white oil emulsion for citrus whitefly control.

PROCEDURE.—All experiments were conducted under lathhouse conditions on 1-year-old liners of *Gardenia jasminoides* v. *radicans* or v. *mystery* plants which had been transplanted into 6-inch metal pots containing approximately 0.1 cu. ft. of soil of uniform texture and composition. Treatments were replicated four times and randomized; from two to four plants constituted a replicate.

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Table 1.—Average number of citrus whitefly nymphs per leaf on *Gardenia jasminoides* v. *radicans* following foliage application of insecticides on October 6 and 30, 1954.

Treatment	Rate/100 Gal. Water	Average No. of Whitefly Nymphs per Leaf on Dates Shown			
		10/29/54	12/17/54	2/17/55	4/5/55
Untreated	—	31.5	22.1	14.1	7.4
Demeton, 50%, E.C.	1 pt.	4.6	1.3	0.1	0.1
Methyl demeton, 50%, E.C.	1 pt.	18.6	5.7	5.2	1.4
Schradan, 90%, E.C.	2 pt.	21.4	6.2	7.0	1.1
Am. Cyanamid 12008, 48%, E.C.	1 pt.	2.6	0.3	0.1	0.0
Am. Cyanamid 12009, 48%, E.C.	1 pt.	4.3	0.5	0.2	0.1
Malathion, W.P., 25%	6 lb.	12.5	4.3	1.2	0.4
Parathion, W.P., 15%	3.3 lb.	13.5	2.7	1.5	0.2
Isolan, 25%, E.C.	2 pt.	17.2	13.5	13.9	1.4
White oil, emulsion	2 gal.	0.6	0.0	0.0	0.0
L.S.D. at 5% level		7.2	4.6	5.7	1.6
at 1% level		9.7	6.2	7.7	2.1

Table 3.—Average number of citrus whitefly nymphs per square inch of leaf on *Gardenia jasminoides* v. *mystery* following soil applications of insecticides on May 3, 1956.

Treatment[a]	Lb. Technical/A.	Average No. Whitefly Nymphs per Square Inch of Leaf on Dates Shown			
		5/24	6/13	7/4	7/28
Untreated	—	9.3	4.3	15.0	19.0
Thimet, dust	1	2.5	0.3	6.8	16.5
Thimet, dust	2	0.5	0.0	7.5	31.5
Thimet, dust	4	0.3	0.0	4.0	14.3
Di-Syston, dust	1	6.5	5.5	8.8	20.5
Di-Syston, dust	2	7.0	3.5	8.8	31.5
Di-Syston, dust	4	2.5	1.0	7.3	30.8
L.S.D. at 5% level		4.6	4.1	none	none
at 1% level		6.3	none	none	none

[a] Insecticides were mixed with the potting medium at the indicated rates per acre of soil to a 6-inch depth.

Foliage applications of several organophosphates were made with a 5-gallon power sprayer. Plants were sprayed to the point of run-off from the leaf surface and special care was taken to spray the lower surface of leaves. All insecticides were mixed with water for application. Soil applications were made by pouring 50 ml. of water containing 0.125% technical insecticide around the base of each plant in each treatment. In one experiment, insecticidal dusts were mixed with the potting medium prior to transplanting the liners. Plants were watered in the same manner after treatment as before treatment. The insecticides used, rates, methods, and interval of application are given in tables 1, 2, and 3.

Whitefly infestations were determined by counting the live nymphs on two leaves per plant. From 16 to 32 leaves per treatment were examined under a binocular microscope and the average number of whitefly nymphs per leaf used to evaluate the effectiveness of treatment. In one experiment, the nymphs per square inch of leaf surface were counted to determine the degree of infestation. Leaves examined were selected for uniformity in size and from about the same position on the plants.

RESULTS.—The results of an experiment on control of the citrus whitefly on gardenia with foliage sprays of several insecticides are given in table 1. About 3 weeks after the first application of insecticidal sprays, all treated plants had significantly fewer whitefly nymphs than the untreated plants. Demeton, Am. Cyanamid 12008 (*O,O*-diethyl *S*-isopropylthio)methyl phosphorodithioate), Am. Cyanamid 12009 (*O,O*-diethyl *S*-(propylthio)methyl phosphorodithioate), and white oil emulsion were significantly more effective for whitefly control than parathion, malathion, schradan, methyl demeton, or Isolan® (dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate). However, there were no differences in the effectiveness of parathion, malathion, demeton, Am. Cyanamid 12008, Am. Cyanamid 12009, and white oil emulsion after the second foliage spray (table 1, column 12/17). Four to 5 months after the second application of insecticidal sprays, nymph populations on all treated plants except those receiving Isolan were significantly lower than on the untreated plants. Infestations on plants treated with malathion, demeton, Am. Cyanamid 12008, Am. Cyanamid 12009, and white oil emulsion were significantly lower than those on plants treated with schradan (table 1, column 2/17). The population level of whitefly nymphs on untreated plants decreased gradually from October to April because of the emergence of adult whiteflies and mortality of nymphs from natural causes.

A comparison of the effectiveness of several organophosphates when applied to the soil or foliage for whitefly control is shown in table 2. Following the application of

Table 2.—Average number of citrus whitefly nymphs per leaf on *Gardenia jasminoides* v. *mystery* following foliage or soil applications of insecticides, 1955.

Treatment[a]	Rate/100 Gal. Water	Average No. Whitefly Nymphs per Leaf on Dates Shown						
		7/22	8/30	9/9	9/22	10/5	10/27	11/18
Untreated	—	4.8	24.9	30.4	31.1	52.6	24.6	48.3
Demeton, 50%, E.C., foliage	1 pt.	11.8	18.6	7.8	2.3	1.4	4.1	2.3
Demeton, 50%, E.C., soil	1 pt.	21.3	59.3	12.5	6.3	2.5	1.5	0.9
Methyl demeton, 50%, E.C., foliage	1 pt.	30.4	46.8	13.8	2.7	2.7	7.5	8.6
Am. Cyanamid 12008, 48%, E.C., foliage	1 pt.	9.4	42.1	3.7	0.8	1.7	8.6	10.4
Am. Cyanamid 12008, 48%, E.C., soil	1 pt.	0.0	1.6	6.8	7.8	6.2	15.4	19.4
Am. Cyanamid 12009, 48%, E.C., foliage	1 pt.	0.4	2.8	11.2	14.2	10.4	19.8	30.8
Delnav, 25%, E.C., foliage	1 pt.	26.9	25.9	14.1	7.1	3.2	4.0	9.2
Delnav, 25%, E.C., foliage	2 pt.	32.8	55.3	25.5	5.2	1.6	3.4	1.9
Parathion, W.P., 15%, foliage	2 lb.	48.6	47.8	8.8	1.4	2.4	11.1	9.9
L.S.D. at 5% level		13.8	25.1	12.5	6.4	5.9	8.9	13.2
at 1% level		18.6	33.9	16.8	8.6	7.7	12.1	18.8

[a] Initial treatments applied March 2, 1955; second application made to all plants exclusive of Am. Cyanamid 12008 and 12009 soil treatments on August 30, third application made to all plants receiving foliage sprays on September 9.

insecticides on March 2, nymph populations on both treated and untreated plants were negligible until July. The population buildup on check plants and plants receiving soil and foliage applications of Am. Cyanamid 12008 and soil applications of Am. Cyanamid 12009 was considerably slower than on all other plants. By the end of August, infestations on all plants except those treated with soil drenches of Am. Cyanamid 12008 and 12009 were sufficiently high to require additional applications of insecticides. Two additional applications of all foliage sprays (August 30 and September 9) and one of demeton to the soil (August 30) were necessary to effectively control whiteflies. Approximately 9 months after initial treatment, whiteflies on plants receiving soil applications of Am. Cyanamid 12008 and Am. Cyanamid 12009 were still significantly fewer than those on untreated check plants. At the termination of the experiment, two soil applications of demeton were equally as effective for whitefly control as three foliage sprays of demeton, methyl demeton, Am. Cyanamid 12008, Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), or parathion, and no significant differences in effectiveness occurred among the chemicals applied as foliage sprays.

Gardenia liners transplanted in potting medium containing 1, 2, or 4 pounds technical per acre of Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) in dust or 4 pounds of Di-syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) dust had significantly fewer whitefly nymphs per square inch of leaf surface 3 weeks after treatment than the untreated check plants (table 3). The 2- and 4-pound rates of Thimet were significantly effective for approximately 6 weeks. The whitefly infestation was somewhat less on all treated plants than on untreated for as long as 2 months after application of insecticides; however, the differences were not significant.

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A Collecting Technique for Pink Bollworms and Other Insects Using a Berlese Funnel with an Improved Heater[1]

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ABSTRACT

Diapause pink bollworm (*Pectinophora gossypiella* (Saund.)), larvae were activated prior to the Berlese funnel treatment by exposing moist, infested material to 80° to 100° F. for 70 to 140 hours. This activation did not necessarily break the diapause. Through the use of heat, larvae of nondiapause pink bollworm, boll weevil (*Anthonomus grandis* Boh.), greater wax moth (*Galleria mellonella* (L.)), a phorid fly (*Megaselia* sp.), and saprophagous flies were forced out of infested material with little difficulty. A heater using a Nichrome wire grid which gave uniform heat was devised for large Berlese funnels, thus allowing large amounts of insect-infested material to be processed with high larval yield.

There has long been a need for an efficient means of removing larvae of the diapause pink bollworm (*Pectinophora gossypiella* (Saund.)) from cottonseed, or similar material, for experimental purposes. For many years larvae have been removed manually by cutting the seed, but this process is slow and tedious and, even with caution, injuries to the larvae cannot be avoided.

It has been observed that the pink bollworm has a negative thermotactic reaction. For example, when infested cottonseed was placed on a white tarpaulin and exposed to sunlight, the larvae abandoned the seed for a cooler area. It was decided, therefore, to conduct experiments at the pink bollworm laboratory at Brownsville, Texas, utilizing heat, which would bring about a mass exodus of the larvae from seed.

A temperature differential to drive insects from their habitat niches has been used for many years, being first utilized by Berlese (1905). Most of the devices developed for this purpose, called Berlese funnels, consist of a heating unit, a funnel-shaped unit to hold the infested material and direct the insects, and a collecting unit. The heating unit is the only one that has changed appreciably through the years. The first one consisted of a water jacket heated by a gas burner (Berlese 1905, Howard 1906, Jarvis 1908, Banks 1909, Wilson 1911). As electrical power became available, the cumbersome jacket was discarded and a simple lid or box holding either a light bulb or a heater, such as a flatiron or an inversion heater, was adopted (Tullgren 1917, Tragardh 1933, Donohoe *et al.* 1934, Christenson 1936).

MATERIALS USED.—Infested seed cotton, cottonseed, and green cotton bolls were used in these experiments. Hand examination showed that there were 100 to 130 pink bollworm larvae per pound of seed cotton, 150 to 200 larvae per pound of cottonseed, and 3 to 10 larvae per green boll. These green bolls were also heavily infested with boll weevils (*Anthonomus grandis* Boh.) and, when rotten, with phorid flies. The seed cotton was picked from fields heavily infested with pink bollworms in the fall and was stored at 55° to 60° F. At this temperature it is possible to maintain healthy inactive larvae within the seed for at least 2 years. The cottonseed was ginned from this seed cotton.

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[2] Resigned August 1, 1958.

[3] In cooperation with the Texas Agricultural Experiment Station.

One-half or 1-pound samples were used for the tests. After each treatment the larvae remaining in the sample and those forced out were counted and the percentage of the total that were forced out was calculated as the yield.

PRELIMINARY EXPERIMENTS.—These experiments showed that pink bollworm larvae can be forced from seeds by the use of heat from one of three sources—a 110-watt incandescent bulb, a 75-watt spotlight, and a 250-watt infrared bulb. Inactive larvae must be activated by moistening the refrigerated seeds and holding them for at least 24 hours. If dry or moist seeds were treated without conditioning, the yield of larvae was only about 3% to 5%. No useful phototactic response was observed.

DESCRIPTION OF HEATING APPARATUS.—An apparatus was designed to give uniform heat over the area being treated (fig. 1). It is 30×30×51 inches high and consists of a heater box resting on a funnel support. The box has a volume of 7 cubic feet. It contains a drawer with a ¼-inch mesh bottom and will hold 4 pounds of cottonseed spread to the depth of 2 inches. It has two parallel grid heaters made of B. & S. No. 32 Nichrome wire, the main one having a rating of 470 watts and the auxiliary 334 watts, both controlled by a chicken-brooder thermostat in which acetone is substituted for the ether in the temperature-actuated wafer. It is not necessary to install two parallel circuits, but an auxiliary circuit is convenient for heating the box rapidly. When the box has reached the desired temperature, the auxiliary circuit can be turned off.

The grid-type heater used in this Berlese apparatus has several advantages over heaters commonly used. These advantages are even heat distribution, ease of control, and ease of design; and they become more important as the size of the apparatus increases. In other methods the use of electric-light bulbs, immersion heaters, and similar devices, although adequate for small collecting apparatus, resulted in such spotty heating that all control was lost.

The heater wire may be mounted in two ways. For small boxes of approximately 1 cubic foot, the Nichrome wire can be strung through holes bored in the wooden frame, thus avoiding insulators. However, to keep the temperature of the wire below the charring point of the wood, the total resistance of the wire must not be less than 100 ohms.

An alternative method is to fasten the wire to ¼-inch asbestos wall board, which is attached to the top of the heater box. This heating system is simpler inasmuch as the wire can be operated at red heat. It may be wired to have two heater units, both controlled by a DPDT switch—either a series circuit or, by center tapping the resistance wire, two parallel circuits, the former unit having one-fourth the wattage of the latter unit.

The wattage needed for the apparatus was determined from the volume of the heater box and frame. However, this wattage was difficult to estimate. The tendency is to install too large a heater, which results in its being on a small portion of the time and causing large fluctuations around the desired temperature. As a general rule, for the best performance the on-off cycling of a heater should be about 50:50. It was found that at a room temperature of about 80° F. the required wattage to maintain 110° could be estimated roughly by the formula $P=60\,V-25$, where P is the wattage and V the volume.

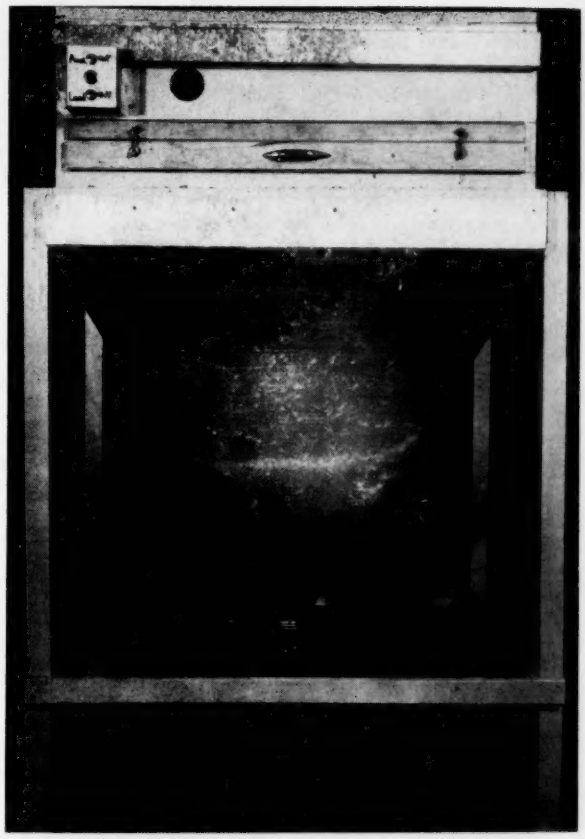

FIG. 1.—Berlese funnel with heating apparatus for collecting pink bollworm larvae.

EXPERIMENTS WITH NEW APPARATUS.—Experiments were conducted in which cottonseed or seed cotton was held under different levels of moisture for various periods up to 168 hours in a greenhouse or an emergence room in which the temperature was controlled at 85° to 95° F. and the relative humidity at 70% to 90% (Richmond & Husman 1957). The most effective moist conditions were determined as follows: (1) Soaking the material in water for various periods of time, (2) placing wet material over damp sand or vermiculite, (3) placing wet material between layers of damp sand or vermiculite, and (4) placing dry material between layers of wet sand or vermiculite. This infested material was placed in the Berlese apparatus and treated at 100° to 120° F. for periods from 30 minutes to 8 hours. Green cotton material was placed in the apparatus with no prior treatment, except that the bolls were opened with a knife or a boll cracker (Clark & Netterville 1957).

Three hundred tests were carried out in developing this technique. Briefly, it was found that 80% to 100% of the diapause pink bollworm larvae could be driven from cottonseed and seed cotton by holding the material under moist conditions for 70 to 100 hours in either the emergence room or greenhouse at 90° to 100° F., and then subjecting it to approximately 110° for 30 to 60 minutes on 3 consecutive days in the Berlese funnel.

High larval yield was dependent primarily upon three

factors—time, moisture, and temperature. Below the 70-hour activation period the number forced out dwindled rapidly; for 50 hours the average yield was approximately 30%. The maximum period of activation was around 140 hours, for after this period the diapause tended to be broken and pupation occurred. The time required for thoroughly activating the larvae varied inversely with their physiological age—the older a larva the more readily it became active.

The same relationship also existed with the time required to force the larvae from the seed. Ordinarily 90% of 18-month-old larvae left in the first 30 minutes, whereas three treatments were need to drive out those 6 months old. The older larvae were less vigorous, smaller, and less resistant to adverse conditions. Although they were easier to activate, they also had a higher mortality. Poor activation or heating the cottonseed too rapidly caused a high mortality of the 18-month-old larvae but a very low mortality of those 3 to 6 months old.

Both the amount of water and the type of agent used to maintain moisture were important, as high moisture conditions favored excessive rot in the infested material, which subsequently brought about a high larval mortality. The most favorable moisture conditions for activation were brought about by sandwiching the cottonseed in plastic screen bags between 4-inch layers of moistened vermiculite. Vermiculite was found to be the best agent because of its high water retention, and as it caused little rot of seeds. Of the various types of sand tried, fine sand was the best; builder's sand had a tendency to bring about rapid decomposition.

The daytime temperatures most favorable for activation were between 90° and 100° F., and the nighttime 80° and 90°, but fluctuations between 80° and 100° did not seem to alter the results. Larval yields above 80% were evidently due to several factors in addition to time, moisture, and temperature. Many experiments were carried out in an attempt to identify these factors, but without concrete results. However, it became more difficult to drive the inactive larvae from the seed as the yield approached 100%.

During the Berlese funnel treatment it was important not to expose the larvae to temperatures above 115° F. Between 115° and 120° heat rigor developed and, although the larvae did not die, they were unable to move. Temperatures of 125° and above for 30 minutes are lethal (Clark 1957).

Nondiapause larvae in all stages were driven from the fruiting forms of cotton. A high percentage of the third- and fourth-instar larvae were recovered, but unless certain precautions were taken most of the first two instars were lost owing to heat sensitivity during the exposure in the device or later through cannibalism.

The larvae of a phorid fly (*Megaselia* sp.) and other saprophagous flies and boll weevils were also driven out of cracked green bolls, between 90% and 100% of the former insect. Boll weevil larvae, as could be expected, were slow in their exodus and more efficient and rapid removal would require a special technique.

This apparatus was also tried on cultures of the greater wax moth (*Galleria mellonella* (L.)) that were used in the insect pathology laboratory at Brownsville. The wax moths were reared in a medium composed mainly of Pablum and honey, and considerable time was consumed in removing the larvae for treatment or storage. The larvae, both free and in cocoons, were forced from the medium when it was spread out in the Berlese funnel.

As a method to obtain pink bollworm larvae in which a uniform high yield is of little consequence, this technique is quite adequate. However, if the goal is a high uniform yield for use in infestation determinations, more study is needed as there are many dependent variables that need further examination.

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SCIENTIFIC NOTES

Evaluation of Off-Odor in Malathion-Treated Wheat[1]

DAVID W. WALKER AND RUTH LOCKE[2]

Malathion has given excellent results when used as a protective treatment on stored wheat in laboratory tests (Watters, 1959). High mortality has been recorded against adults of the saw-toothed grain beetle. *Oryzaephilus surinamensis* (L.); flat grain beetle, *Laemophloeus pusillus* (Schönk.), and lesser grain borer, *Rhizopertha dominica* (F.), with treatments as low as 0.1 p.p.m. Similar results have been obtained for other species of stored-grain insects when this material was used as a contact insecticide (Vincent & Lindgren 1957).

However, objectionable off-odors frequently were found in milling grain and in baking with flour milled from wheat treated with standard technical grade malathion. Germination has not been affected and there has been no off-flavor in bread made with flour milled from treated samples of wheat in previous tests (Walker 1957).

PROCEDURES.—Work was undertaken to determine whether malathion odor was detectable at 8 p.p.m., the recommended dosage of this material. One series of treatments was made using fresh, premium grade malathion emulsifiable concentrate at 4, 8, 16 and 100 p.p.m. levels 68 days before evaluating odor, and another series 1 day prior to evaluating.

Grading System.—A panel was set up for eight samples. A covered Pyrex double boiler, with water boiling in the lower portion and 500 grams of wheat in the upper portion, was used for each sample. The samples were kept hot at all times and replaced frequently with a fresh aliquot from the same lot of wheat.

One sample was identified as an untreated control. Panel members were asked to smell the control first, and as often as they wished between samples, to acquaint themselves with normal wheat odors. The other samples included one treated with 4 p.p.m. malathion and stored 68 days before evaluation; two samples treated with 8 p.p.m., one stored 68 days before evaluation and the second, 1 day; one sample treated with 16 p.p.m. and stored 68 days; two samples treated with 100 p.p.m., one stored 68 days and the second, 1 day; and one sample of untreated wheat from the same lot as the control sample.

Odor was described as "objectionable" or "not objectionable" and graded on a scale of 1 to 10 points difference from the untreated control. Scores of 1 and 2 for an individual sample for a panel member indicated no difference from the known control; 3 and 4—a slight difference; 5 and 6—a moderate difference; 7 and 8—a large difference; and 9 and 10—an extreme difference. Thus a minimum possible score for one treatment by the 28 panel members of between 28 and 56 would show no difference from the untreated sample. A maximum possible score of 252 to 280 would show an extreme difference.

The order of evaluations was a randomized block design and the sequence was changed for each evaluator. Scoring was done by the evaluator and a separate record was kept for each. Tabulated results were analyzed by the Duncan (1957) Multiple Range Analysis method.

RESULTS.—The results of the evaluations are given in table 1. There was no significant difference among treatments at 0 to 16 p.p.m., regardless of the time interval between formulating and evaluation. The 100-p.p.m. treatments differed significantly in odor from the 16-p.p.m. treatment made 68 days before, and from the 8-p.p.m. treatment made 1 day before the evaluation ($p=.01$). Sixty-eight per cent of the evaluators judged the 100-p.p.m. treatment, stored 1 day, as "objectionable." Eighty-four per cent found the 100-p.p.m. treatment, stored 68 days, "objectionable." These results indicate that an application of premium grade malathion at the recommended rate of 8 p.p.m. for storage periods of 68 days, will not produce an objectionable odor in wheat.

Table 1.—Results of evaluation of off-odor in malathion-treated wheat.

TREATMENT (P.P.M.)	DURATION OF EXPOSURE (DAYS)	TOTAL SCORE
4	68	96
Untreated	1	97
8	68	106
16	68	116
8	1	119
100	1	176
100	68	197

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A Field Test for the Control of *Trichoplusia ni* (Hbn.) on Celery with Several Insecticides and *Bacillus thuringiensis* Berliner[1]

A. A. GRIGARICK[2] and Y. TANADA[3,4]

Populations of the cabbage looper (*Trichoplusia ni* (Hbn.)) occur in peak densities on celery in San Joaquin County, California, during late September and October. A field test for the control of the cabbage looper was initiated in this area on October 7, 1958. Several experimental and currently used chemical insecticides and two formulations of a microbial insecticide containing spores of *Bacillus thuringiensis* were included in the test.

METHODS.—The check and 10 treatments were replicated four times in a randomized complete block design. Each of the replicated plots measured 25×50 feet. The materials were applied with Hudson hand sprayers and dusters at a rate of 50 gallons of water or 50 pounds of dust per acre. A wetting agent, Colloidal X-77 (Colloidal Products Corp., San Francisco, California), was added at the rate of 1:1500 to all the spray treatments. The celery plants were 10 to 15 inches high when

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Table 1.—Results of treatments for the control of *Trichoplusia ni* on celery.

Treatment	Formulation and Concentration	Active Ingredient per Acre	Mean Number of Larvae per 25 Feet of Row			Mean Number of 3rd–5th Instar Larvae per 25 Feet of Row		
			Days Following Treatment[a]					
			3	7	14	3	7	14
Malathion+Perthane	4%+5% dust	2.0+2.5 lb.	5[a]	4[a]	16[a]	4[a]	2[ab]	1[a]
DDT+Toxaphene+Sulfur	5%+15%+45% dust	2.5+7.5+20 lb.	7[ab]	14[b]	20[a]	1[a]	0[a]	1[a]
Thiodan	3% dust	1.5 lb.	17[c]	25[c]	62[b]	1[b]	7[b]	5[b]
Bacillus thuringiensis	3×10^9 spores per gm. dust	6.8×10^{13} spores	31[d]	37[c]	46[b]	9[b]	1[a]	0[a]
Parathion	2% dust	1.0 lb.	12[c]	27[c]	108[c]	10[b]	5[b]	5[b]
Phosdrin	1.5% dust	0.75 lb.	12[bc]	60[d]	163[cd]	9[b]	7[b]	4[b]
Dimethoate	4 lb./gal. EC	1.0 lb.	84[ef]	78[de]	135[cd]	23[c]	21[c]	32[d]
Bacillus thuringiensis	5 lb./100 gal. of 60×10^9 spores/gm.	6.8×10^{13} spores	101[efg]	101[def]	168[cd]	18[bc]	5[b]	21[c]
Dibrom	4% dust	2.0 lb.	62[e]	128[fg]	216[d]	21[c]	27[c]	17[c]
Am. Cyanamid 18706[b]	2 lb./gal. EC	1.0 lb.	115[fg]	129[efg]	193[d]	30[c]	30[c]	33[d]
Check	—	—	147[g]	193[g]	221[d]	23[c]	34[c]	37[d]

[a] Analyses were made on transformed data. Treatments associated with the same letter are not significantly different from each other at the 5% level.
[b] *O,O*-dimethyl *S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate.

treated and they were harvested approximately 60 days thereafter. There were approximately 40 plants per 25 feet of row.

Counts of looper larvae were made at intervals of 3, 7, and 14 days after treatment. The larvae were recovered by vigorously shaking the plants over an insect collecting net (a closely woven muslin bag 15 inches in diameter) as the net was moved through a 25-foot row of celery plants within each replicated plot. Larvae collected in this manner were classified according to their approximate instars. Inasmuch as some of the chemicals were less toxic to the more mature larvae, the data were tabulated in two categories: first- to fifth-instar larvae, and third- to fifth-instar larvae only.

Results.—Although the field containing the test plots had been previously treated on September 8 with a combination of DDT and toxaphene, the looper infestation, which consisted mainly of immature larvae, was high at the beginning of the present test (October 7). The check population showed 85% of the loopers to be in the first and second instars when the counts were made on the third day following treatment; 82% in these instars after 7 days; and 83% after 14 days. Because of the continuous oviposition by the cabbage looper adults during this period, it was necessary to treat the field again the third week following the application of the test materials in order to save the celery crop.

The looper counts showed varying degrees of favorable control for 6 of the 10 test materials (table 1). Two factors favored a high level of control. The plants were sufficiently small to be adequately covered with insecticides and the looper population consisted predominantly of early instar larvae. Several of the chemicals gave somewhat poorer results in a previous experiment (unpublished 1957) because both plants and loopers were more mature at the time of application.

The two dust combinations, malathion+Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane) and DDT+toxaphene +sulfur, yielded the highest control. The larval counts showed that these treatments were effective against all instars for at least 14 days. The inclusion of sulfur in the DDT-toxaphene mixture was incidental since this mixture was not available without sulfur at the time of the test.

Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), *B. thuringiensis*, parathion, and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) dusts were on a somewhat lower plateau of control. Parathion and Phosdrin produced the highest reduction in larvae in the earlier counts, but the toxicity of Thiodan and *B. thuringiensis* dusts apparently persisted for a longer period. The *B. thurgingiensis* dust compared favorably with the malathion+Perthane and DDT+toxaphene dust combinations in effectiveness against the third- to fifth-instar larvae. The relatively high counts of first- and second-instar larvae in the *Bacillus* plots do not necessarily indicate a resistance to the bacterial by these instars, but more likely to their limited feeding on the lower leaf surfaces where the application of the spores was inadequate. *B. thuringiensis* applied as a spray did not approach the level of control attained when it was applied as a dust. The reason for failure was attributed to differences in plant coverage since the spore count was the same for the two formulations. Hall & Andres (1959) also reported the superiority of dust over spray formulations of *B. thuringiensis* spores.

The cabbage looper was continuously destructive up to the harvest of celery in this area and the need for preharvest control introduces residue problems. The absence of toxicity to plants and mammals by *B. thuringiensis* (Steinhaus 1957) strongly suggests a potential usage of this organism for looper control on celery. Other workers (Tanada 1956, Hall & Andres, 1959) have reported partial or effective control with *B. thuringiensis* against the cabbage looper on broccoli, cabbage, and cauliflower. In the case of the cabbage looper on celery, additional investigation with the *Bacillus* is necessary to determine the most effective dosage and methods of application. The possible use of *B. thuringiensis* in combination with a chemical insecticide also needs investigating because the selectivity of the pathogen may not be adequate for the control of other insect pests, especially sucking insects, on celery.

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Polybutenes—a New Control for Phytophagous Mites[1]

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The increasing difficulty of controlling phytophagous mites by chemical means stresses the need of finding new approaches and of re-examining control principles that were discarded with the appearance of powerful chemical acaricides. This note describes the use of a polybutene to kill mites by mechanical means, somewhat similar to the use of bill-posters' paste by Hutson (1936).

A number of Indopol Polybutenes[2] varying in viscosity from 40 to 20,500 S.S.U. at 210° F. were examined for acaricidal properties against the two-spotted spider mite, *Tetranychus telarius* (L.), on several greenhouse crops. Indopol H-1500 (viscosity 15,000 sec.) a very sticky, light amber material, showed remarkable promise for trapping active stages. One hundred ml. of this polybutene, 100 ml. water, and 3 ml. Emulsifier G-3300[3] were emulsified with an electric stirrer. This stock was diluted with water to give a 5% emulsion. When sprayed onto foliage heavily infested with mites, the polybutene was deposited as minute sticky spheres in the webbing and as sticky flattened droplets on exposed leaf. Motile forms of the mite were immobilized at once. Eggs hit by the spray were killed; those protected by the webbing hatched, but the larvae were trapped and killed by the droplets as soon as they began to move about the web. As the polybutenes are nonvolatile and insoluble in water, they should have a long residual action.

Melon plants growing on wires in a greenhouse and heavily webbed by a massive infestation of mites were sprayed with 5% polybutene emulsion. Control of the mites was complete by the time all the eggs had hatched, and subsequent plant growth was free of mites. Excellent control of the mite was also given by the same treatment on rose, honeysuckle, peach seedlings, and other plants in the greenhouse.

Spraying of uninfested plants with emulsions of Indopol H-1500 also prevented or delayed subsequent infestations by the two-spotted spider mite. Halves of lima bean leaves were sprayed with the emulsions and the other halves protected from the spray; the untreated halves were then heavily infested with active mites of all stages. A 5% emulsion allowed no invasion of the treated area at 7 days and only a very small marginal invasion at 11 days (fig. 1). A 3% emulsion allowed a small marginal invasion at 7 days, which increased to about 25% of the treated area at 11 days. A 1% emulsion offered little resistance, the treated area being 50% infested at 7 days and 95% at 11 days. The mites eventually entered the areas treated with the 3% and 5% emulsions by spinning webbing over the surface, and became established in patches, presumably where the spray deposit was least. Halves of leaves, sprayed with the emulsifier only, became quickly and uniformly infested.

FIG. 1.—Lima bean plant 7 days after half of each leaf had been sprayed with 5% polybutene emulsion and the unsprayed half infested with two-spotted spider mite. Injury causes infested halves to appear paler.

No phytotoxicity resulted from any of the treatments, even on young, succulent growth.

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[1] Accepted for publication April 30, 1959.

[2] Amoco Chemical Corp., 910 South Michigan Ave., Chicago, Illinois; distributed in Canada by R. J. Brown Co., 150 Bronoco Ave., Toronto, Ontario, Canada.

[3] Atlas Powder Co. of Canada, Ltd., Brantford, Ont.

A Note on the Persistence of Phosdrin[1]

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How long does Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), or a plant metabolite of Phosdrin, remain toxic to insects in the leaves of a deciduous tree? A recent study conducted at the Central States Forest Experiment Station in Columbus, Ohio, revealed the toxic period to be 2 to 3 days.

METHODS.—Twenty-five larvae of the orange-striped oakworm *Anisota senatoria* (J. E. Smith) were placed on each of 24 pin oak *Quercus palustris* (Muenchh.) saplings (1½ to 2 inches in diameter and 8 to 10 feet tall) and allowed to migrate to the foliage. Three days later the insecticide was applied directly to the bark of these trees with a pipette. No attempt was made to "ring" the tree, but care was taken to prevent the insecticide from running off. The bioassay organisms were a laboratory culture of daphnids *Daphnia pulex* (De Geer) and field-collected larvae of the orange-striped oakworm. Treatments consisted of four replications of 0.5-ml., 1-ml., and 2-ml. doses of technical Phosdrin placed directly on the tree trunks. Observations were made twice daily following treatment: at 8:00 A.M. and at 4:00 P.M.

During each morning observation four 5-gram, green-weight foliage samples were taken from each tree. Each sample was placed in 20 ml. of distilled water and blended for 5 minutes in a Waring blendor. Next, the sample was centrifuged for 5 minutes, filtered and the filtrate divided into two 10-ml. portions. Ten daphnids were placed in each of the 10-ml. portions and mortality recorded.

For each observation at 24-hour intervals following treatment 1,920 daphnids and 600 oakworms were used for determining the toxicity of the leaf samples.

RESULTS.—*24 Hours After Treatment.*—Daphnid reactions to the 1-ml. and 2-ml. treatments compared favorably with a one-part-in-five-million of technical Phosdrin in distilled water; reactions to the 0.5-ml treatment compared favorably with a one-part-in-ten-million solution of technical material in water. All the oakworm larvae appeared "normal" and were active with

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the exception of two that fell from a 0.5-ml.-treatment tree and three that fell from two untreated trees.

48 Hours After Treatment.—A bioassay with daphnids revealed toxicity comparable with a one-part-in-ten-million for the 1-ml. and 2-ml. treatments; no differences were evident between the 0.5-ml. treatments and the check trees. Some oakworm mortality was observed (table 1).

Table 1.—Mortality of oakworm larvae 48 hours and 72 hours after treatment with Phosdrin.

Dose (ml.)	Number of Larvae		
	Dead	Living	Not Recovered
	48 hours after treatment		
0.5	28	68	4
1	44	56	0
2	58	42	0
Check	13	281	6
	72 hours after treatment		
0.5	95	0	5
1	97	0	3
2	97	0	3
Check	15	271	14

72 Hours After Treatment.—The daphnid reactions showed differences between untreated and treated trees. Oakworm kill appeared to be complete (table 1).

Further Observations.—Bioassay with daphnids continued for 3 days after the oakworms had died or dropped from the treated trees, but no toxicant was detected. Of the 300 oakworms on the untreated check trees, 223 of the larvae continued feeding and pupated.

Throughout the test no evidence appeared of damage to the foliage, but the treated trunk area became dotted with small spots of dead bark, 1 to 1.5 cm. in diameter. These spots were more common near the point of application.

From the experiment it would appear that Phosdrin or its metabolites persist in leaf tissue of pin oaks for a period of more than 48 but less than 72 hours following a trunk application of the technical material. There is also a lag of at least 24 hours in the mortality of orange-striped oakworm larvae, when compared with daphnids.

Evaluation of Damage to Cotton Caused by Low-Clearance General-Purpose Tractor in Applying Insecticides[1]

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Entomological research has provided the cotton grower with basic information on the proper selection and most economical usage of insecticides and spraying and dusting equipment. However, less attention has been given to the means by which application equipment is propelled over the field. Specialized equipment is preferred on large acreages of rank cotton. Such equipment includes the airplane, high-clearance self-propelled duster or sprayer, and high-clearance general-purpose tractor equipped with fenders. Although the means of propulsion are many and varied, economics should be considered in the selection of equipment for a particular area.

Without specialized equipment, growers have a tendency to compare the expected damage from the use of available equipment with the expected increase in yield from the use of insecticides. Many growers in North Carolina are reluctant to treat rank cotton, even when insect control is greatly needed, because of the expected severe injury to the crop by ordinary equipment. This factor appeared to have been a contributory cause of a loss of approximately 65% of the cotton crop to the late brood of bollworm (*Heliothis zea* (Boddie)) in the Central Coastal Plain during 1955.

Theoretically, it is possible to show that the damage inflicted by ordinary machinery has been overemphasized in relation to the benefits that may be expected from chemical control of severe insect infestations. However, no field data on this subject are available.

Damage to rank cotton caused by a low-clearance general-purpose tractor in applying insecticides for control of a severe boll weevil (*Anthonomus grandis* Boh.) infestation was evaluated in 1956. The results reported herein were obtained on a typical small farm in Robeson County, North Carolina.

Materials and Methods.—The experiment consisted of nine replicates. Each replicate consisted of 2 tractor rows and 10 boom rows and covered ½ acre. Three replicates were dusted with 2% dieldrin-5% DDT, three with 2½% methyl parathion-5% DDT, and three with 2½% Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate)-5% DDT. Adjacent outside check plots, each 12 rows wide, covering ½ acre, were maintained on two opposite sides of the test field.

Commercially available machinery was used. A 6-row duster was mounted on the rear of a 4-wheel tractor and operated from a 10-inch pulley on the power takeoff. The boom was used 4 inches above the tops of the plants, with one nozzle directly over each 42-inch row. The tractor straddled a single row, with the main body of the tractor directly over the cotton plants. The tractor was not equipped with protective shields. Ground clearance of the tractor on a level surface was 13 inches. The tractor was driven at approximately 5 m.p.h. over rows 3 and 10 of each replicate in the same direction throughout the test.

The test field was planted to Coker 100W cotton on April 13 and fertilized and cultivated in accordance with recommended practices. Rainfall was well distributed throughout the growing season. On July 11, boll weevils of the first generation had punctured 70% of the squares. Eleven applications of dust, at an average rate of 21.3 pounds per acre and an average interval of 3.3 days, were made from July 11 to August 15.

Infestation records were taken at weekly intervals from the six center rows of each dusted replicate and check plot. Average plant height was 4.5 feet. Foliage was interlaced between rows, but there was no lodging. Squaring ended in all plots about August 10. Yield records were taken from tractor rows, six center boom rows of each dusted replicate, and six center rows of each check plot. The cotton was harvested on September 12 and October 16 without the aid of chemical defoliation.

Results.—The average square infestation of dusted replicates was 61%, while the average infestation of check plots was 98%. The average number of adult weevils per 100 squares in dusted replicates was 7.5, while the average number of weevils in check plots was 18.9. Infestations of other pests were negligible throughout the season.

Yields in pounds of seed cotton per acre were as follows: adjacent outside check 898, tractor rows, 1,172, and boom rows 1,438. An analysis of tractor rows vs. boom rows showed that the following yield differences were required for significance: at the 5% level 148, and at the 1% level 232.

The tractor rows produced 266 pounds of seed cotton per acre less than the boom rows and 274 pounds more than did untreated rows. The tractor rows comprised one-sixth of the total treated area. The yield increase resulting from the use of insecticides was thus reduced by 49% on the rows that the tractor straddled and 8% over the entire treated area. The loss of seed cotton

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which could be attributed directly to the use of the tractor is applying insecticides amounted to 44 pounds per acre.

The average yield from the entire treated area was 1,394 pounds of seed cotton per acre, which was 496 pounds more than from the untreated area. The actual increase in yield resulting from the use of the equipment and insecticides described herein amounted to 55%, whereas, if the tractor had not damaged the crop, the increase would have been 60%.

Effects of Physical Properties of Derris Dusts on Their Toxicity to the Mexican Bean Beetle[1]

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One of the problems in controlling the Mexican bean beetle (*Epilachna varivestis* Muls.) with derris dusts has been the variation in effectiveness of dusts from different sources. To obtain a better understanding of the reasons for this variation, tests were conducted at Columbus, Ohio in 1943 to determine the effects of different properties of derris dusts on their toxicity to the beetle. Earlier tests had shown that their toxicity to the pea aphid (*Macrosiphum pisi* (Harr.)) did not increase with the derris content but did increase with the greater fineness of grinding of the derris root and with the decrease in particle size of the diluent (Harries 1956).

Four samples of derris root of different rotenone content were each ground to three degrees of fineness and diluted to a strength of 1% of rotenone with pyrophyllite of four degrees of fineness. The 48 dusts were compared in a 4×3×4 factorial experiment to study the effects of differences in each of the major factors and their possible interaction effects. Eight replicates were made. Greenhouse plants in the large two-leaf stage were transplanted into No. 2 tin cans, treated with insecticides under a bell-jar duster (Harries 1943), infested with 10 third-instar larvae, and covered with cylindrical screen cages with cellulose acetate tops to admit as much light as possible. The cages were then placed on a table under fluorescent lights at 80° F. and 50% relative humidity. After 3 days the numbers of dead and moribund larvae were recorded and the leaf area eaten was estimated by placing a transparent grid over the leaves and counting the squares where more than half the area was consumed. The particle size of the derris samples and the pyrophyllite was determined as surface-mean diameter with an air permeation apparatus (Gooden & Smith 1940).

The data are summarized in table 1. Both the larval mortality and the leaf area eaten show highly significant effects of the percentage and particle size of the derris root and the particle size of the diluent. The dust with the highest percentage of derris root was significantly less toxic than dusts with less root. Also, the derris samples of 6 and 7 microns were significantly more toxic than the 9-micron sample. There was a decrease in toxicity with increase in particle size of the pyrophyllite, and the 3-micron diluent produced a significantly more toxic dust than any of the coarser ones.

Presumably, greater fineness of milling of the derris root could affect the toxicity by more thorough breaking down of the plant structure to expose the contained rotenone and/or by increasing the number of particles and surface area of the root encountered by the insects. If the latter effect were the more important, the toxicity could also be expected to increase with the derris content of the dust. Since there was no such indication in the data, the breakdown of the plant structure appears to be the more important effect. The highly significant interaction between fineness of grinding and the percentage of ground root shows that derris of low rotenone content was most improved by finer milling. Similar results were obtained in tests of the same dusts against the pea aphid (Harries 1956).

Table 1.—Effects of the derris content and the particle sizes of the derris and pyrophyllite on the toxicity of 1% rotenone dusts to the Mexican bean beetle.

PROPERTY	PER CENT MORTALITY OF LARVAE	LEAF AREA EATEN (SQ. CM.)
Derris content (per cent)		
8.6	74	36
11.1	79	29
15.4	79	30
25.6	68	46
L.S.D. at 5% level	7	9
Particle size of derris (microns)		
6	76	33
7	78	26
9	70	46
L.S.D. at 5% level	5	6
Particle size of pyrophyllite (microns)		
3	82	29
6	75	35
9	72	37
12	70	40
L.S.D. at 5% level	6	7

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Effectiveness of Three Organophosphorous Compounds against Larvae of the Painted Lady in Alfalfa[1]

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Migrations of the painted lady or thistle butterfly, *Vanessa cardui* (L.), occur at irregular intervals in southern California (Abbott 1941, 1946, 1950, Sudgen *et al.* 1947, Woodbury *et al.* 1942). A heavy migration of *V. cardui* took place during the winter of 1958.

Painted lady larvae feed on a variety of species in the genera *Malva* L., *Arctium* L., *Lupinus* L., *Urtica* L., and species in the Cynoreae (thistle) tribe. The larvae are frequently a pest on ornamentals, especially chrysanthemum and hollyhock, and they are sometimes found on oak trees where they rarely, if ever, cause damage. When populations occur in large numbers they may attack globe artichoke (=genus *Cynora*), beans, sunflower, prune trees, lettuce, cotton, and okra.

During February and March 1958, painted lady larvae were extremely abundant in alfalfa fields in the Imperial Valley, where they were feeding principally upon cheeseweed, a wild mallow (*Malva parviflora* L.). In many instances, the caterpillars com-

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pletely defoliated the mallow plants and began feeding on the alfalfa, where the larval populations were extremely heavy. In only a few cases, however, did the larvae cause damage of economic importance to the alfalfa. This occurrence of large numbers of painted lady larvae in alfalfa fields provided an opportunity to test the effectiveness of three organophosphorous insecticides against this sporadic pest.

In general, the larval infestations were not uniform in the alfalfa because the mallow grew in scattered areas. In one case a 30-acre field of newly seeded alfalfa was heavily infested with mallow, and painted lady larvae occurred quite uniformly throughout the field. On March 3, 1958, parathion, Phosdrin®, (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), and Dylox®[2] (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) sprays were applied by aircraft to the alfalfa field. Each material was applied at the rate of 4 ounces of toxicant per acre. Two replicates were made of each treatment, and each replicate was 90 feet wide and 1,225 feet long. Each compound was applied using 5 gallons of spray liquid per acre. The alfalfa was about 12 to 15 inches tall.

Table 1.—Effect of parathion, Phosdrin, and Dylox sprays applied by aircraft for control of painted lady larvae in alfalfa in the Imperial Valley, California, on March 3, 1958.[a]

	2 Days		5 Days	
Material[b]	Total Larvae	Per Cent Mortality	Total Larvae	Per Cent Mortality
Parathion	502	92	25	99
Phosdrin	408	93	108	97
Dylox	1227	79	907	73
Untreated	6003	—	3367[c]	—

[a] Number of larvae and per cent mortality based on 200 net sweeps at indicated intervals following treatment.
[b] Four ounces of active ingredient applied in 5 gallons of water per acre.
[c] Reduction in larval population was due to pupation.

The effect of the chemicals was evaluated 2 and 5 days after application by sampling with a standard insect sweep-net. Five-sweep samples were made at 20 locations in each replicate, or a total of 40 5-sweep samples per treatment. Each sweep described an 180° arc in the alfalfa. The data are summarized in table 1.

Two days after application parathion, Phosdrin, and Dylox gave 92, 93, and 79% mortality of the painted lady larvae, respectively, in comparison with the untreated plots. Five days after application the respective mortalities obtained with these materials were 99, 97, and 73%. The data show that parathion and Phosdrin gave satisfactory control of *Vanessa cardui* larvae. On the other hand, Dylox applied at 4 ounces per acre failed to give satisfactory control. For successful results somewhat higher dosages of this latter material are indicated.

At the time of application most of the caterpillars were of medium to large size, a range representative of late third, fourth, and early fifth instar larvae. When the second sample was taken, 5 days after application, it was observed that many of the older larvae remaining in the treated plots had fallen or crawled from the plants and pupated, or attempted to pupate. However, a very high percentage of these pupae were dead and had begun to decompose. In the untreated plots large numbers of larvae had successfully pupated, and the pupae reacted with vigorous movements when probed with a pencil. This pupation accounts for the decrease in larval populations in the untreated plots when the latter were sampled 5 days after application.

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[2] Known also as Bayer L 13/59 and Dipterex. Produced by Farbenfabriken Bayer, Leverkusen, Germany, and furnished by Chemagro Corporation, Kansas City, Missouri.

Inositol: An Indispensable Dietary Requirement for the Boll Weevil[1]

Erma S. Vanderzant, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*, in cooperation with the *Texas Agricultural Experiment Station*[2]

In previous rearing studies (Vanderzant & Davich 1958) it was found that the boll weevil (*Anthonomus grandis* Boh.) did not grow on a purified diet containing casein as the protein but developed if casein was replaced by soybean protein, Drackett assay C-1, or if the casein diet was supplemented with yeast extract or brewer's yeast. These yeast preparations gave growth that varied with different batches. Inositol was suspected as the growth factor missing in the casein, since soybean protein and yeast contain small amounts of it. A purified diet then was devised that was satisfactory for growth of the weevil to the adult stage. This diet also permitted the determination of the quantitative nutritional requirements for myo-inositol, designated simply as inositol, since none of the other eight isomers are known to have any significant biological activity. The composition of the diet was as follows:

Constituent	*Grams*
Casein, vitamin-free (Nutritional Biochemicals Corp.)	5.0
L-Cysteine·HCl	.1
Sucrose	5.0
Corn oil (Mazola)	.25
Cholesterol	.05
Salts, Wesson's	1.0
Choline chloride	.1
Inositol	.1
Sodium alginate (Kelcosol)	.5
Agar (Difco Bacto granules)	3.0
	Milligrams
Zinc acetate dihydrate	.5
Cobalt chloride hexahydrate	.25
Sodium molybdate dihydrate	.25
Niacinamide	1.0
Calcium pantothenate	1.0
Thiamine·HCl	.25
Riboflavin	.5
Pyridoxine·HCl	.25
Folic acid	.25
Biotin	.02
Vitamin B_{12}	.002

2M potassium hydroxide to pH 6.5
Water to 100 grams

The preparation of the diet and eggs and the rearing conditions were essentially the same as described previously (Vanderzant &

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[2] This work was partially supported by funds from the Foundation for Cotton Research and Education.

Davich 1958). Aseptic conditions were used throughout these studies. Eggs were obtained from the 15th and 16th generations of Mexican weevils reared from egg to adult on artificial diets and fed squares as adults.

Development on the casein diet with various amounts of inositol is shown in table 1. In the absence of inositol, the larvae died in the first instar or soon after the first molt. No pupae or weevils were reared on a diet containing less than 1 mg. of inositol per 100 grams. Growth was slow at concentrations of 1 to 5 mg. A concentration of 7.5 mg. appeared to be sufficient, because the weights, rates of development, and yields were not improved by increasing amounts of inositol up to 100 mg. per 100 grams of diet.

Table 1.—Development of the boll weevil on a casein diet with various concentrations of myo-inositol.

Milligrams of Inositol per 100 Grams of Diet	Number of Larvae	Days to First Pupa	Number of Adults	Average Weight of Adults (Mg.)	Per Cent Yield of Adults
0	142	—	0	—	—
0.1	61	—	0	—	—
0.5	39	—	0	—	—
1	106	25	5	2.9	4.7
2	53	17	5	5.7	9.4
5	113	11	75	7.0	66
7.5	104	10	84	8.1	81
10	105	10	86	8.2	82
20	101	10	67	8.2	66
40	52	10	40	8.4	77
60	107	10	79	9.1	74
80	43	10	33	8.8	74
100	586	10	429	9.0	73

Twenty adults with an average weight of 5.3 mg. also were reared on a modification of the amino acid diet that was developed for the pink bollworm (Vanderzant 1957). Inositol was added and corn oil was omitted from this diet. No growth was obtained without the inositol. Preliminary results indicate that the larvae also can use combined forms of inositol. When calcium phytate and acetone-insoluble soybean phosphatides were substituted for inositol, good growth was obtained.

An absolute dietary requirement for inositol has not yet been demonstrated for any animal, although a beneficial effect on growth has been reported for several insects and vertebrates. Fraenkel & Blewett (1943) showed that the beetles *Tribolium confusum* Duv. and *Ptinus tectus* Boieldieu grew better when the diet contained inositol. The growth of *Ephestia kuehniella* Zeller also appeared to be improved by inositol (Fraenkel & Blewett 1947). Forgash (1958) found that inositol was necessary for the maturation of the American cockroach (*Periplaneta americana* L.). Under certain dietary conditions several vertebrates show a need for inositol (Lardy 1954). In all these studies synthesis of inositol by either the animal or its symbiotes was not ruled out.

Under aseptic conditions inositol has been shown to be an indispensable nutrient for the boll weevil and the amounts needed lie in the same range as those of the water-soluble vitamins.

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An Electrically Operated Micro Dispenser[1]

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A number of devices for accurate delivery of small quantities of insecticide have been described in the past. Most of these dispensing mechanisms are modifications of the micrometer-controlled syringe of Trevan (1922). By use of a ratchet system attached to the micrometer, the investigator need not refer constantly to the vernier scale, as delivery of a constant volume is assured by a proper stop setting. However, manual operation is still required.

A dispensing device which permits operation without manual activation is described below. All the operator need do is position the insect beneath the delivery needle and press a foot switch. Both hands are left free and attention can be focused on the

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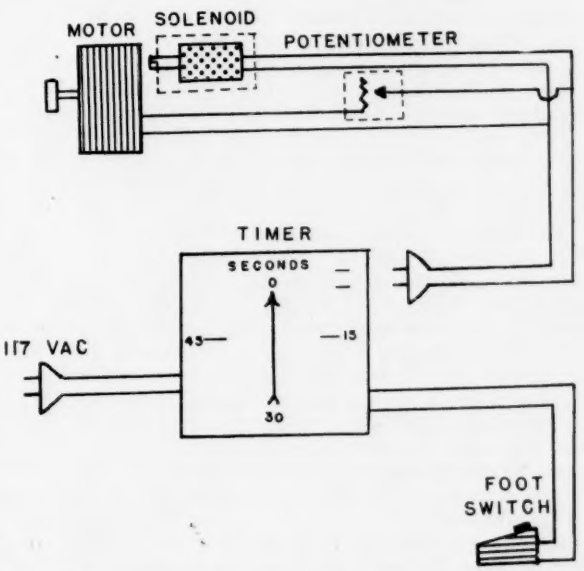

Fig. 1.—Wiring diagram of the electrical micro dispenser. The sections within the dotted lines represent optional accessory modifications (see text for full descriptions).

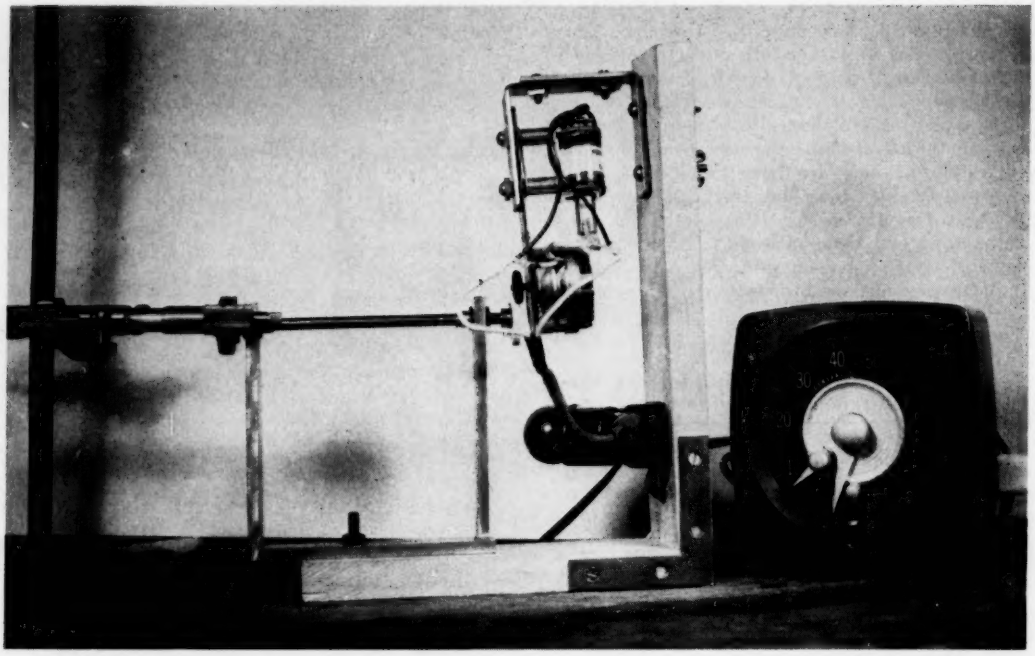

FIG. 2.—Overall view of the electrically operated micro dispenser. Micrometer (left), motor and solenoid above (center), timer (right).

subject. This device may be an asset to the toxicologist who must work alone and requires speed of operation. The basic mechanism of this dispenser is a synchronous motor operated through a timer which rotates a micrometer for a pre-set number of seconds. Total cost for all parts exclusive of the timer was about $6. The timer cost varies from 10 to 25 dollars for a new timer, or less for a used one.

The equipment consists of a 1½ r.p.m. synchronous motor, a 110 volt AC continuous duty solenoid and a resetting photographic timer. The timer is actuated through a foot switch which allows motor rotation for a selected time interval.

Figure 1 illustrates a wiring diagram of the delivery apparatus. The solenoid and potentiometer are accessories which may be added at the discretion of the operator. The solenoid is wired in parallel with the motor and is actuated with it. As soon as the current is switched off by the timer, the solenoid plunger is released and instantaneously brakes the motor. A potentiometer may be used to vary the speed of the motor, if desired. The power rating and resistance value of the potentiometer are dependent on the size of the motor, in this case four watts and 1,000 ohms, respectively.

The dispenser is illustrated in figure 2. Note that the solenoid is placed above the motor flywheel. Gravity carries the plunger downward against the flywheel as soon as the current is switched off. A ½-inch diameter rubber wheel is fixed to the motor driveshaft. A ¼-inch diameter extension shaft, 6 inches long, with 1-inch diameter wheels at both ends, transmits motor rotation to the micrometer handle. The purpose of the extension shaft is to allow clearance for the micrometer handle. Thus by the use of a larger diameter driveshaft wheel or by employing a motor with an offset shaft, micrometer clearance is accomplished. Any increase in output speed resulting from a larger driveshaft wheel may be compensated for by a proper potentiometer setting. Both the micrometer handle and extension shaft are friction driven although geared unit modifications may be used in place of the friction drive. The unit's housing and desk clamps will depend on conditions encountered in individual laboratories.

The timer may be any one of several photographic resetting timers currently on the market. Some units are factory equipped with foot switch outlets, while in others a simple wiring addition to the manual switch is required.

The micrometer and syringe are calibrated in the usual manner with either water or mercury. Note is made of the number of divisions of the vernier scale required to deliver the desired dose. The timer is then adjusted so that this number of divisions is exactly rotated by the motor. From this point, all that is necessary is actuation of the timer by the foot switch.

It was found that for a 1-microliter dose, 0.005 inch of the micrometer travel was required. Delivery error from 100 trial doses was less than 0.005% per dose, or 1 microliter ±.005.

A list of materials appears below. All electronic parts may be procured from Burstein-Applebee Co., Kansas City, Mo. Prices apply to these items as found in their latest catalog. Resetting photographic timers may be obtained from any photographic outlet. One timer, manufactured by the Heath Company, Benton Harbor, Michigan, comes in kit form. This timer is completely electronic with no moving parts. The cost of this timer is considerably below that of factory-made models. Accessories, such as wires, plugs, and outlets, may be obtained locally from specialty stores.

Quantity	*Item*	*Cost*
1	Motor, synchronous, 1½ r.p.m., 110 VAC.	$1.95
1	Solenoid, 110 VAC, continuous duty	2.48
1	Potentiometer, 1,000 ohms @ 4 watts	1.12
1	Extension shaft, 6 inch, ¼ inch diameter with bearing	.48
2	Flexible couplers (use as 1 inch wheels)	.96
1	Foot switch (home or factory made)	variable
1	Resetting photographic timer	variable

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Millipede Injury to Potatoes[1]

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The millipede *Blaniulus guttulatus* Bosc. was first observed in a garden at Union Gap, Wash., in 1943 and has been found since at various places in central Washington, where it attacks irrigated crops. Injury is frequently confused with that caused by flea beetle larvae and wireworms. The millipede is particularly annoying as a contaminant in fresh produce.

B. guttulatus was introduced into the United States from Europe many years ago and is present in all parts of the country that have a long cultivation history (Chamberlin 1921). In Europe it infests beans, cabbage, cucumber, peas, potatoes, strawberry, sugar beet (Rolfe, 1937), carnation (C. J. G. 1914), chrysanthemum, onions, tomato (Anonymous 1934), corn (Uvarov 1914), garlic, hyacinth, pumpkin, rape, tulip, turnip, wheat (Anonymous 1914), gourd (Chamberlin 1921), grape (Poisson 1925), hop (Theobald 1914), lettuce (Schroyen 1914), mushrooms (Austin 1933), and rutabaga (Pape 1941).

B. guttulatus appears to be the most common millipede pest of potatoes in Europe and the northwestern part of the United States. Rolfe (1937) reported that, of 130 cases of millipede damage recorded in England and Wales from 1923 to 1936, 65 referred definitely to *guttulatus*. Blunck (1933) stated that many crops of potatoes in Schleswig-Holstein were destroyed in 1932 by millipedes, including *guttulatus*. Wilson (1943) found that, although *guttulatus* is usually a secondary pest found in soil rich in organic matter, when abundant it will feed on the skin of soft-skinned varieties of potatoes, and the injuries facilitate the invasion of bacteria and fungi. MacLeod (1935), working with another species, *Diploiulus londinensis* var. *caeruleocinctus* Wood, in New York, reported that there was less injury to the Russett Burbank than smooth-skinned varieties.

These two millipedes are very similar in appearance. Each is about 15 mm. in length, 1 mm. in width, and has from 55 to 75 pairs of legs. However, *guttulatus* is lighter brown with white legs and has about 35 prominent dark reddish-brown, ovate spots on each side of the body (fig. 1), whereas *caeruleocinctus* has a mottled brown body.

B. guttulatus was observed infesting the remnants of old potato seed pieces in a garden at Union Gap in April 1943, and tubers of the new crop harvested from a shaded part of the garden were found infested in July. Light injury to tubers was observed nearly every year in gardens or heavily composted fields in various parts of eastern Washington. It was unusually abundant in 1949 when it was found infesting carrots, lily bulbst potatoes, and tomato fruit that touched the ground. In 1952 i,

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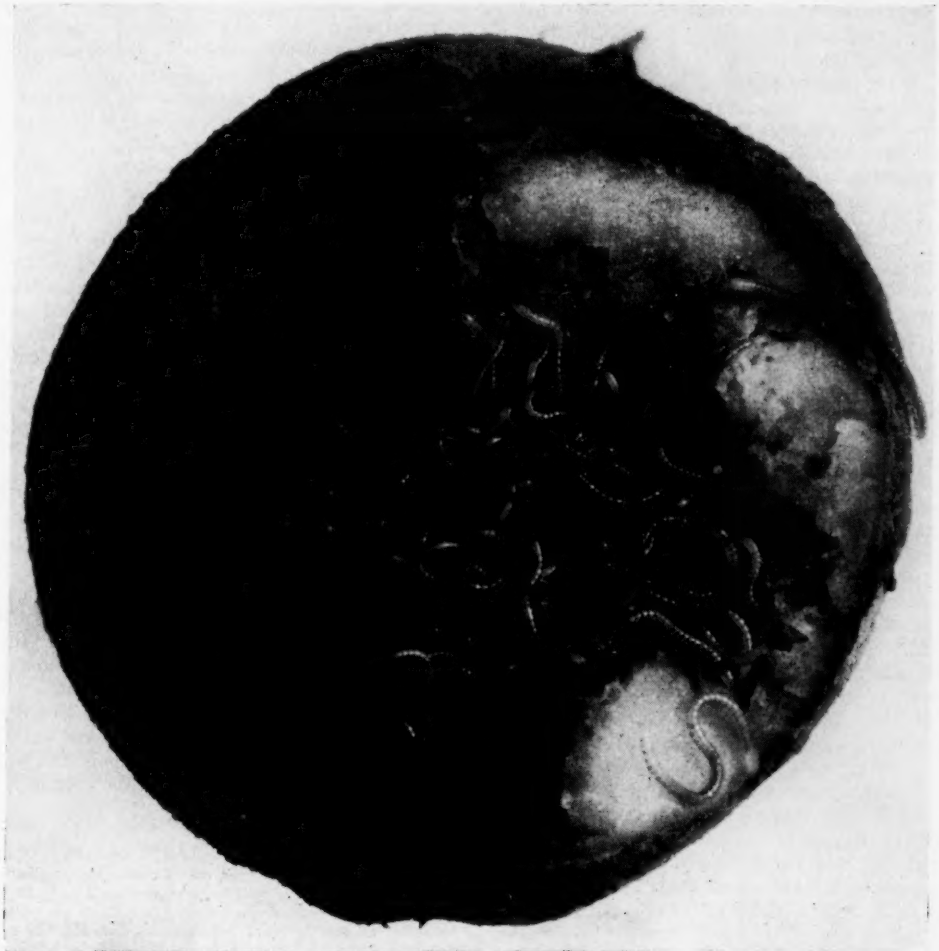

FIG .1.—The millipede, *Blaniulus guttulatus* Bosc., infesting a potato tuber that overwintered in the soil.

was found infesting asparagus roots, and a few individuals had tunneled into the basal portion of the spears, which were later harvested. In 1959 it was collected from soil in a greenhouse bench.

Millipedes have been observed overwintering in potato tubers and other decayed vegetables, where from 50 to several hundred sometimes occurred together in a gently roiling mass. They seldom appear on the soil surface, since they require a humid environment. The millipede is probably the most important pest of potato seed pieces in gardens and commercial fields during cool, wet springs.

B. guttulatus makes cylindrical, clear-cut tunnels 1.5 to 2 mm. in diameter in potato tubers or seed pieces. The tunnels are bored in a straight line and may be as much as 3.8 cm. long. They retain a fresh appearance, and little discolored scar tissue is formed, whereas tunnels produced by flea beetle larvae are soon bordered with a thick layer of tough, dark brown scar tissue, and wireworm tunnels are usually black on the inside because of granular black frass.

In a control experiment in 1945 freshly cut Russet Burbank seed pieces were dipped in a paste made of DDT or phenothiazine mixed with flour and water. All the plants made some growth, and on May 30 they were carefully lifted and the seed pieces and the soil around the root masses were examined. From 32 to 37% of the seed pieces had been infested with millipedes, irrespective of treatment. However, in the soil adhering to the roots, there were 14 living millipedes per hill following treatment with paste containing 0.4% of DDT as compared with 27 for flour alone. In the DDT plots 66% of the millipedes were dead. Only 16 living millipedes were found following treatment with a flour paste containing 4% of a commercial phenothiazine preparation.

In 1947 BHC, at the rate of 1 pound of the gamma isomer per acre, was thoroughly mixed in the soil in one section of the garden before the potatoes were planted. No millipede injury was observed at harvest-time, although slight injury was observed in untreated soil. However, in 1948 millipedes were again abundant in the part of the garden that had received the BHC treatment.

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Field Observations of Increasing Resistance of Cattle to Cattle Grubs[1]

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The use of systemic insecticides to control cattle grubs, *Hypoderma* spp., for the past several years has resulted in data that suggest the possibility of immunity or resistance of cattle to cattle grubs. Cattle, treated for grubs one season, appear to have fewer grubs the following season with no further treatment.

It is common knowledge among livestock men that bovine calves and yearlings harbor more cattle grubs than older animals. Peter & Gaehtgens (1934) reported that cattle more than 5 years of age were observed to be free from grubs. Hadwen & Fulton (1924) reported that a natural resistance to cattle grub invasion increases with the age of the animal. Scharff (1950) reported that Tretsven (1943), in an unpublished report, experimented with calves exposed to heel flies for two seasons, and found a decreased number of grubs the second season. Tretsven attributed this to a naturally acquired immunity. Scharff also reported that natural resistance of cattle to grubs varies widely and that in some heavily infested herds there will be individuals present that are free from grubs. On 53 animals observed, Scharff reported average natural grub mortality amounted to 35%. In four other herds observed, he reported natural grub mortality to range from 19 to 73%.

Since there are indications that there is natural resistance or immunity built up against cattle grubs, it seems probable that when all the grubs in an animal are killed, there should be an even greater antibody production causing a greater resistance or immunity.

With this in mind, two experiments were conducted at Hays, Kansas to determine if grubs killed in the backs of animals produced resistance to future infestation.

The first experiment was conducted on 86 Hereford steer calves acquired in October from New Mexico. In November of 1956, 41 were bolused with Trolene®, (*O,O*-dimethyl *O*(2,4,5-trichlorophenyl)phosphorothioate, formerly designated Dow ET-57) and 45 were used as untreated controls. All had grubs in the back at the time of treatment. The average number of live grubs per head during the winter of 1956–57, based on accumulative counts, was 3.5 for the treated; 21.4 for the untreated controls. These same steer calves were held over through the next year with no further treatment. The previously treated steers the following winter (1957–58) had an average of 0.9 grub per head as compared with 3.3 grubs per head for the untreated controls.

The second experiment was conducted on 82 Hereford steer calves, acquired in October from the same place as the calves in experiment one. Dowco 109 (*O*-(4-tertiary butyl-2 chlorophenyl), *O*-methyl methyl phosphoramidothioate) was used during November of 1957. Twenty-eight calves were sprayed, 28 were bolused, and 26 were used as controls. Again all calves had grubs in the back at the time of treating. The average number of grubs per head found on the sprayed animals after treatment during the winter of 1957–58, was 3.8; the average found in bolused animals after treatment was 1.8 grubs per head and in untreated animals, 41.4. One year following (1958–59), with no further treatment, the previously bolused animals had an average of 9.3 grubs per head and the previously sprayed animals, 12.8 compared with 16.4 for the untreated animals. The possibility of the spray not killing as many grubs in the animals' backs may ex-

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Gratitude is expressed to Stuart Ratcliff for his generous help with this study.

plain the higher grub count on the sprayed animals the following year.

The seasonal difference in rate of infestation of the control animals is probably the result of the adult heel fly population density, *i.e.*, the population density of the adult fly is greater in New Mexico than in Kansas. Consequently, the data on the average number of grubs on the untreated control steers for the first season are not comparable with those found the following year.

These observations made on animals treated one season and observed the following season indicate an increased resistance to the grubs by the animals. The amount of resistance seems to be the result of the number of grubs in the host body at the time of treatment. Thus, for an animal having a high number of grubs killed in its body, indications are that the animal would have fewer or no grubs the following year. Also, an animal having few grubs killed in its body could be expected to have the same or a larger number of grubs the following year. Some animals, depending on the degree of infestation, may need two or more seasonal treatments before an increased resistance to grubs is shown.

Studies of this nature are still under way on Hereford steer calves that have had 99% of the grubs killed during the winter of 1958–59. These steers will be available for further grub counts during 1959–60.

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Effect of Alfalfa Variety on the Biology of the Spotted Alfalfa Aphid in Arizona[1]

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The release of Lahontan and Moapa alfalfa for commercial use in western United States has necessitated a study of the biology of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) on these varieties. Both are resistant to the aphid, but Lahontan, a winter-dormant type, is recommended for cold areas and Moapa, a nondormant type, for areas in the Southwest. In 1957 the senior author observed damage to seedlings of these varieties in Arizona under heavy aphid populations in the field, from 25 to 42% of the Lahontan plants and 23 to 44% of Moapa being killed. Under the same conditions, however, stands of other varieties—Chilean 21-5, New Mexico 11-1, Pilca Butta, Caliverde, Gactula, California Common, Saladena, Peru Alkali Resistant, Hairy Peruvian, DuPuits, and Indian—were completely killed.

The adverse effect of different plants on the biology of insects has been summarized by Painter (1951, 1958), who classified it as antibiosis or one of the mechanisms of resistance. Harvey & Hackerott (1956) observed that seedling plants of alfalfa varieties affected the survival and reproduction of the spotted alfalfa aphid. Dobson & Watts (1957) reported fewer aphids on certain alfalfa varieties, and Howe & Smith (1957) found a high mortality of aphid nymphs on resistant varieties.

Several life history studies on the spotted alfalfa aphid have already been reported. Dickson *et al.* (1955) gave an account of the aphid's life history in California, but did not mention the variety of host alfalfa. In Israel, Harpaz (1955) stated that no significant difference in the life history was found between two varieties of clover and one of alfalfa. Nielson & Barnes (1957) used Chilean 21-5 in their study in Arizona. The aphid's biology in Kansas on different species of legumes was reported by Peters & Painter (1958).

At Mesa, Arizona, lath-house studies were made in 1958 to compare the effects of two resistant (Lahontan and Moapa) and two susceptible (Chilean 21-5 and African) varieties of alfalfa on the life history, fecundity, and longevity of this aphid. All plants were infested with aphids in the unifoliolate stage on June 4, and the study was terminated on July 8 when they were approximately 18 inches tall.

Life histories were determined by placing a single apterous female on 10 plants of each variety grown singly in 5-inch pots and determining the length of each instar of the progeny from daily observations. Results of this study are shown in table 1. The aphid required about 6½ days to complete its life cycle on Chilean 21-5 and African, and 1 and 2 days longer on Moapa and Lahontan, respectively. The extended period of nymphal development on resistant varieties generally conforms with results obtained by Dahms (1948) on the chinch bug and Harrington (1941) on the pea aphid. On the susceptible varieties the first three instars were of about the same length. On Lahontan, however, the time increased with each instar except the fourth, but on Moapa it was somewhat variable. Much higher percentages of nymphs developed into adults on Chilean 21-5 and African than on Moapa and Lahontan. Fewer adults survived and pro-

[1] Accepted for publication June 12, 1959.

[2] In cooperation with the University of Arizona Agricultural Experiment Station.

Table 1.—Effect of alfalfa variety on the length of the different instars of the spotted alfalfa aphid and the percentage completing each instar, June 4–July 1, 1958.

VARIETY	NUMBER OF ADULTS	INSTAR	LENGTH (DAYS)	PER CENT OF NYMPHS COMPLETING INSTAR
Chilean 21-5	41	First	1.7	90
		Second	1.8	66
		Third	1.8	51
		Fourth	1.2	46
		Total	6.5	
African	41	First	1.7	83
		Second	1.7	66
		Third	1.7	46
		Fourth	1.1	44
		Total	6.3	—
Moapa	76	First	2.3	29
		Second	2.0	24
		Third	2.1	18
		Fourth	1.2	17
		Total	7.6	—
Lahontan	103	First	1.7	10
		Second	2.3	3
		Third	3.7	3
		Fourth	1.0	2
		Total	8.7	—

Table 2.—Effect of alfalfa variety on longevity of adult spotted alfalfa aphids, the per cent that reproduced, and the number of progeny per day, June 4–July 1, 1958.

Variety	Number of Adults	Average Longevity (Days)	Per Cent of Adults that Produced Progeny	Number of Progeny per Adult per Day
Chilean 21-5	43	13.4	37	3.9
African	55	12.6	33	3.0
Moapa	89	13.0	10	2.8
Lahontan	103	13.3	8	2.4

duced young on Lahontan and Moapa, as indicated by the number of adults tested in comparison with Chilean 21-5 and African. Nymphal mortality was very high on Lahontan but light on Chilean 21-5.

For the studies on fecundity and adult longevity other series of 10 plants of each variety were used, but a single fourth-instar nymph was placed on each plant. Observations were begun as soon as nymphs developed into adults. As shown in table 2, adult longevity was nearly the same, about 2 weeks, on all varieties tested. The number of progeny per adult per day was 1½ times as great on Chilean 21-5 as on Lahontan. The percentages of adults that produced progeny were significantly higher on Chilean 21-5 and African than on Moapa and Lahontan.

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A Modified Pollen Trap for Honey Bee Hives[1]

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In pollen-identification studies conducted at Logan, Utah, samples in the pollen trap often got wet during storms and had to be discarded. Traps similar to the one described by Todd & Bishop (1940) and Scullen (1943) were used until 1949, when a modified trap was tested and found to be superior. Other traps described by Schaefer & Farrar (1941) and Killion (1945) were less adaptable to our needs. In the modified trap the vertical screen grid made of 5-mesh hardware cloth was located underneath the hive near the entrance. As the bees worked their way through the screen barrier, the pollen pellets were knocked off their legs and fell into a tray below. The pollen samples were collected by removing the tray from either the front or the side near the entrance.

The location of the tray near the front of the hive exposed the pollen to frequent dampening by rain or dew. A wooden shade board was improvised to protect the pollen pellets in the tray, but it was not satisfactory. A prototype of the trap herein described was tested during the 1949 and 1950 seasons, and none of the many samples of pollen collected by it were affected by excess moisture. Many of the pollen samples collected at the same time with the older traps were lost. Several of the improved traps, with further modifications, were constructed as illustrated in figure 1.

The double screen grid, mounted in a metal channel to permit its removal, was located beneath the middle of the hive instead of the front. The floor in the entrance to the trap was sloped toward the front of the hive so that water would not run into the tray.

The tray was made slightly smaller than the slot in the side of the trap assembly to allow for expansion during stormy weather. A piece of spring metal 1 inch wide and 3 inches long was fastened to the bottom of the tray to hold it firmly in place. A piece of quarter round was fastened to the side of the trap just above the tray to direct water from it. In addition, a saw cut was made just back of the front panel of the tray and parallel to it to prevent any water getting past the quarter round from running into the tray. Large holes for ventilation were cut in the sides of the trap just back of the tray and covered with hardware cloth. A screened false floor was placed back of the tray for

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[3] In cooperation with the Utah Agricultural Experiment Station.

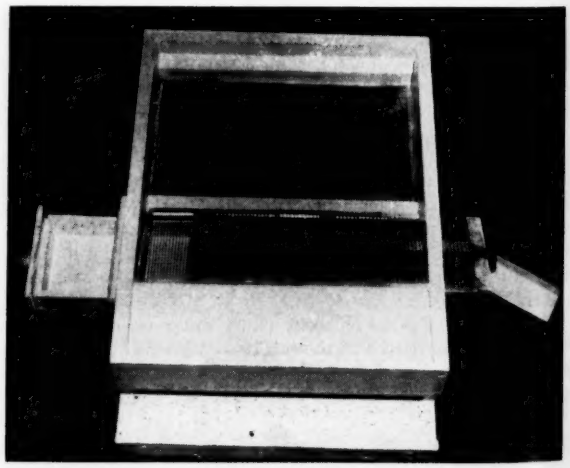

Fig. 2—Illustration of pollen trap.

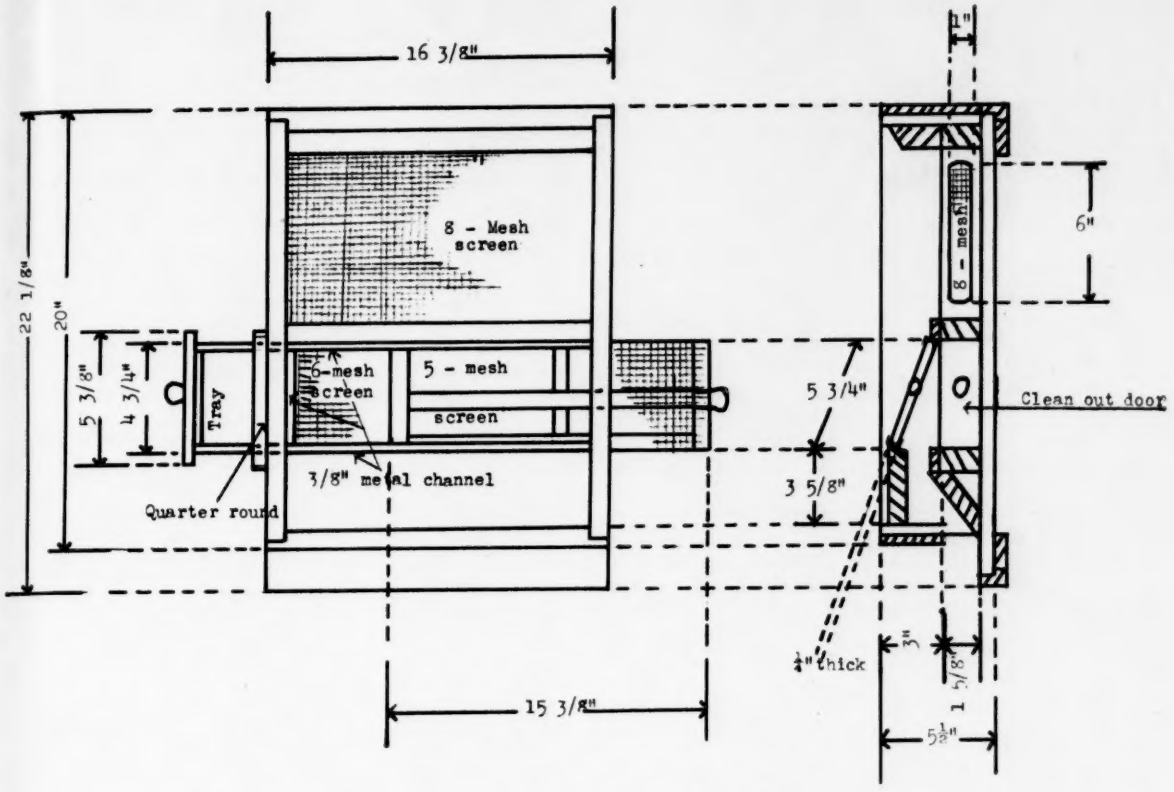

FIG. 1.—Drawing of new pollen trap showing quarter round above tray to keep out rain, and clean out door opposite tray, and removable grid screen.

better ventilation. This false floor also tends to prevent the bees from building comb in the deep space beneath the combs.

The most important feature of the new trap is the protection of the pollen from moisture. It also permits removal of the pollen tray from the side of the hive without disturbing the bees that cluster at the entrance in warm weather (fig. 2). The screen grid, which slopes toward the rear of the hive, is 4 inches wide instead of the usual 2 inches. This extra grid surface provides more rapid passage of the bees and minimizes their clustering at the entrance thus permitting better ventilation of the hive.

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Purple Scale Parasite Found in Florida[1]

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Purple scale, *Lepidosaphes beckii* (Newm.), is the most important scale insect on citrus in Florida, being found in nearly every grove throughout the State. Although the scale is attacked by several species of parasites, predators, and entomogenous fungi, natural control is generally inadequate (Muma 1955).

In June 1958 emergence holes of an unidentified parasitic wasp were observed in mature female purple scales on leaves from an unsprayed citrus grove near Fort Pierce, Fla. (Muma & Clancy 1959). Microscopic examination of living and recently killed scales revealed the presence of eggs, larvae, and pupae of a small golden chalcid. Reared adults from this and subsequent collections in other groves were identified as *Aphytis lepidosaphes* Compere.[2] This parasite was introduced into California from China in 1948–49 (Flanders 1950), and was later sent to Texas and Mexico, but there is no record of its introduction into Florida.

A. lepidosaphes has been found to occur practically throughout peninsular Florida from Monticello in the extreme north to the Homestead area south of Miami, even in isolated plantings far removed from the main citrus areas. It was reared from samples from 37 of 50 properties during July and August, and from 50 of 51 scale samples collected between September 4 and November 10, 1958. These were mostly unsprayed groves, but the parasite also occurs commonly in sprayed groves. Parasitization of adult females, the preferred host stage, has ranged from 0 to 56.3% with an average of 24.6%. The abundance and widespread distribution of this parasite indicate that it has probably been present in Florida for some time, but how and when it was introduced is not known. Its apparent rapid increase following a very severe winter is also an enigma.

A. lepidosaphes is an external parasite which lays its eggs on

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[2] Identified by Harold Compere, California Citrus Experiment Station, Riverside.

either the dorsal or ventral surface of the scale body, usually the latter. Special studies revealed that of the scales containing this parasite 94.8% were adult females, 4.9% were males, and 0.3% were second-instar females. Although young adult females are apparently preferred as hosts, older females in various stages of egg laying are also attacked. The translucent, ovate parasite eggs adhere lightly to the scale body, which seems to be unharmed by the ovipositing parasite. Two or more eggs are often found clumped together, indicating deposition by a single female, and the same scale may be attacked by more than one parasite, as shown by the presence of different stages. However, 52.2% of the parasitized adult females examined had only one parasite, 43.3% had two parasites, 4.1% three parasites, 0.3% four parasites, and 0.1% (1 scale) had five parasites. In cases of gregarious parasitism all adults issue through a single hole in the scale armor cut by the first individual. Host size apparently determines the number that can develop, as males and second-instar females rarely produce more than a single adult parasite.

The larvae of *lepidosaphes* are nearly circular in outline, slightly flattened, and white to pale pinkish in color. Pupae are clear yellow to amber, with distinct red eyes, and the active adults are bright lemon yellow. Of 2,676 adult parasites reared and examined thus far, 58.6% were females and 41.4% were males. In California (Flanders 1950) the life cycle is approximately 15 days, and there are about two parasite generations to each scale generation.

In Florida males and second-instar females are parasitized by *Aspidiotiphagus lounsburyi* (B. & P.), *A. citrinus* (Craw.), and *Prospaltella aurantii* (How.), but these species seldom attack adult females. The addition of *lepidosaphes* to the parasite complex therefore fills an important gap and should result in more effective natural control of purple scale. Studies are being continued to determine the importance of this parasite in relation to other scale enemies.

In a survey of citrus plantings along the Gulf Coast in November 1958, *lepidosaphes* was reared abundantly from purple scale on Satsuma orange leaves collected near Magnolia, Port Sulfur, and Buras in the delta area south of New Orleans, La. Dead scales showing parasite emergence holes were also numerous, as in Florida. This is apparently another new state record with unknown source of introduction. However, there was no evidence of the parasite in several Satsuma plantings near Silverhill and Fairhope, Ala., and it has not yet been recorded from Mississippi, Alabama, or northwestern Florida.

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Control of Resistant Spider Mites on Greenhouse Roses[1]

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One of the major problems on greenhouse roses is the resistance of the two-spotted spider mite (*Tetranychus telarius* (L.)) to acaricides. Many growers have resorted to frequent applications of various mixtures or syringing the plants with water to obtain control of this pest. Experiments were conducted at Beltsville, Maryland, to compare the effectiveness of various acaricides against a highly resistant strain of this spider mite. In 1958 experimental applications were made also in a commercial greenhouse in New Jersey.

EXPERIMENTS AT BELTSVILLE.—Two experiments were conducted on four beds of 3-year-old roses. Each bed was divided into 4 plots—2 plots consisting of 16 plants each of the variety Better Times; 1 plot of 18 plants of Cavalier; and 1 plot of 4 plants each of Talisman and Snow White, 8 each of Briarcliff, and Golden Rapture. The plots were set up in a latin-square design consisting of four treatments each.

The plants were infested with a strain of spider mites that has had a high resistance to all the organic-phosphorous insecticides, Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfiite), and Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol). Previous tests have indicated that a buildup of this strain has been prevented only with weekly applications of a spray containing Aramite plus schradan.

In the first experiment the following wettable powders were used in 100 gallons of water: 1 pound of 50% ovex, 2 pounds of 25% Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical), 2 pounds of 25% Tedion plus 2 pounds of 15% Aramite, and the same quantitity of Aramite in a 1–1,000 dilution of schradan. All sprays contained one-half pint of Santomerse (alkyl aryl sulfonates) as a wetting agent. They were applied with a 2-gallon hand sprayer at 60 pounds pressure, and the nozzle was directed to give thorough coverage of the lower leaf surface. Eight weekly applications were made, the first on December 23, 1957, and the last on February 10, 1958.

Each week counts were made of all living forms on 10 disks, 9/16 inch in diameter, taken at random from leaves showing any feeding damage. The results (table 1) indicate that Aramite plus Tedion or schradan controlled this strain, whereas ovex and Tedion alone were relatively ineffective.

During the third week of February the rose plants were cut back to force new growth from the main canes. This pruning removed practically all the old foliage. Spider mites were transferred to the new growth of each plant and allowed to become established before the second experiment was begun on March 26. The treatments were the same as in the first experiment except that 2 pounds of 15% Aramite plus a 1–1,000 demeton was substituted for the ovex.

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Table 1.—Control of resistant two-spotted spider mites on greenhouse roses. Beltsville, Maryland.

TREATMENT	NUMBER OF MITES PER PLOT AFTER WEEKS SHOWN						
	2	3	4	5	6	7	Mean
	First Experiment						
Ovex	118	89	94	133	140	173	124
Tedion	117	195	163	50	61	312	149
Aramite plus—							
Schradan	3	8	13	29	10	3	10
Tedion	5	2	2	1	1	3	2
	Second Experiment						
	4 Weeks	5 Weeks	6 Weeks	8 Weeks	9 Weeks		
Tedion	65	17	53	10	60		41
Aramite plus—							
Demeton	55	26	27	177	75		72
Schradan	9	1	35	28	7		16
Tedion	3	1	8	15	8		7

Mite counts were made on April 23 and weekly through May 29. The plants sprayed with Aramite plus Tedion or schradan had significantly fewer mites than those sprayed with Aramite plus demeton or Tedion alone (table 1). Most of the spider mite injury on the plants treated with the last two mixtures was confined to the areas that showed injury before the sprays were applied, but injury was evident over most of the new growth of plants receiving Tedion or Aramite plus demeton.

Spray injury was evident in plots treated with the Aramite-schradan combination about 2 weeks after the first application in both experiments and after 4 weeks in the Aramite-demeton plots of the second experiment. This injury was confined mostly to the lower leaves, which developed chlorotic and dark green areas, and later died. The plants in these plots were almost devoid of lower foliage in 6 to 8 weeks.

No obvious spray injury of lower foliage occurred in plots treated with Tedion, ovex, or Aramite-Tedion. Lack of injury from the Aramite-Tedion treatment indicated that either the schradan or the demeton in the combination or their interactions with Aramite caused the phytotoxic effect. To determine the effect of this injury upon flower production, yield data were obtained in all plots in the second experiment during April and May. The mean numbers of flowers per plant were as follows: Tedion 4.8, Aramite-schradan 4.6, Aramite-demeton 5.3, and Aramite-Tedion 6.0.

Although the plants treated with Aramite-schradan had the fewest flowers, this combination was as effective as Aramite-Tedion for controlling spider mites. The loss in yield may be due to the phytotoxic effect on the plants. Subsequent preliminary observations indicated that Aramite, Tedion, or a combination of these materials will cause yellowing of foliage and leaf drop to the White Butterfly variety of roses.

EXPERIMENTS IN COMMERCIAL GREENHOUSE.—Further tests of Aramite and Tedion were conducted against a resistant strain of spider mites on commercial roses at Cranbury, New Jersey. This strain was first established as resistant to organic-phosphorus compounds in 1949 (Smith & Fulton 1951), and the strain on the Beltsville greenhouse roses is a segregate of this Cranbury strain collected in 1949. Since control of this strain has not been obtained on roses at this establishment with any acaricide, the grower had resorted to frequent syringing with water.

On February 14, 1958, a randomized-block design was set up consisting of four treatments replicated three times. Each plot consisted of one bed of roses 150 feet long. The plants of two replicates were of Better Times and one was of Golden Rapture variety.

A factorial arrangement of treatments was as follows: Syringed, Aramite, Tedion, and Aramite plus Tedion. Aramite (15%) and Tedion (25%) wettable powders were used at 1 pound each in 100 gallons of water. All sprays contained one-half pint of Santomerse-S as a wetting agent. They were applied with a greenhouse sprayer at approximately 150 pounds pressure, except the syringed treatment, which was frequently hosed with water through a special nozzle.

Spider mite populations were estimated on April 2 and May 9, about 3 weeks after a series of three weekly applications made in February and March and repeated in April. Ten disk samples were taken at random from leaves showing mite feeding. Most of the lower leaves were gone from plants in the plots that were syringed with water, but the plants of the other treatments were still holding most of the lower leaves.

As shown in table 2, the Aramite-Tedion combination had greatly reduced spider mite populations on April 2. The largest number of mites found on May 9 were in the replicate of Golden Rapture that had very dense foliage. These high populations may have been due to inadequate spray coverage, since the feeding injury was confined to lower leaves in small isolated areas of the plots.

None of the treatments had any apparent toxic effect upon the tolerant varieties of plants, but the syringing caused premature defoliation of the lower leaves. The Aramite-Tedion spray combination has now been used for a full year on the roses in all greenhouses at Cranbury. The only varieties injured were Garnet and Carol Amling by the Aramite component and White Butterfly by Tedion.

Table 2.—Control of resistant two-spotted spider mites on commercial greenhouse roses.

TREATMENT	NUMBER OF MITES PER PLOT		
	April 2	May 9	Mean
Syringed with water	399	449	429
Aramite	120	263	192
Tedion	74	62	68
Aramite plus Tedion	0	130	65

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Toxic Effects of Boron Trioxide against Immature Stages of *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus*[1]

R. W. FAY[2]

In 1928, Matheson and Hinman, using commercial borax solution and *Culex pipiens* L. and *Aedes vexans* (Meig.) as the test species, found that 1.5 grams of borax per liter of water prevented development of larvae for an observation period of 6 weeks.

To determine the immediate toxic effects of boron trioxide solutions against immature mosquitoes, laboratory evaluations were made with eggs, first-, second-, and third-instar larvae, and pupae of *Aedes aegypti*, *Anopheles quadrimaculatus* Say, and *Culex quinquefasciatus* Say. A test concentrate (30,000 p.p.m.) was prepared using 30 grams per liter of metaboric acid ($B_2O_3 \cdot H_2O$) [Fisher Catalogue #A-76, Boric Acid, fused (approximately metaboric acid) Powder Purified], and aliquots of this concentrate were diluted with tap water to obtain the test solutions.

OVIPOSITION, HATCHING, AND FIRST-INSTAR LARVAE.—*Aedes aegypti* females laid eggs that hatched after more than 72 hours in a test solution having 8,000 p.p.m. metaboric acid. Oviposition tests with *A. quadrimaculatus* or *C. quinquefasciatus* adults did not provide conclusive results.

Using eggs previously laid in plain tap water, successful hatching was observed with all three species in test solutions of 2,000, 4,000, and 8,000 p.p.m. metaboric acid. Within 24 hours complete mortality of newly hatched *C. quinquefasciatus* larvae occurred in all three concentrations, of *A. aegypti* in the 4,000 and 8,000 p.p.m. solutions, and of *A. quadrimaculatus* in the 8,000 p.p.m. solution. At 48 hours only *A. quadrimaculatus* larvae persisted in the 2,000 p.p.m. test solution. No mortality was encountered in check larvae.

SECOND- AND THIRD-INSTAR LARVAE.—In tests with second-instar larvae, metaboric acid solutions proved most effective against *A. quadrimaculatus* (table 1). In initial tests with third-instar larvae, the results of 24-hour exposures to various metaboric acid solutions, expressed as combined per cent of dead and

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[2] From the Communicable Disease Center, Public Health Service, Bureau of State Service, Department of Health, Education, and Welfare, Savannah, Georgia. The author gratefully acknowledges the assistance of Mrs. Mary S. Crawford in the performance of the laboratory tests.

Table 1.—Per cent dead and moribund[a] second-instar *Anopheles quadrimaculatus*, *Aedes aegypti* and *Culex quinquefasciatus* larvae with 24- and 48-hour exposures to various metaboric acid ($B_2O_3 \cdot H_2O$) solutions.

	quadrimaculatus		*aegypti*		*quinquefasciatus*	
$B_2O_3 \cdot H_2O$ (p.p.m.)	24-Hr.	48-Hr.	24-Hr.	48-Hr.	24-Hr.	48-Hr.
0	0	0	0	12 (48)	0	4
100	56 (24)	92	—	—	—	—
500	100	100	0	20 (52)	—	—
1,000	—	—	—	—	0	76 (4)
1,500	—	—	36	96 (4)	—	—
2,000	—	—	—	—	84 (16)	100
2,500	100	100	—	—	—	—
3,000	—	—	48	100	—	—
4,000	—	—	—	—	100	100

[a] Figures in parentheses indicate moribund larvae.

moribund larvae, again showed approximately the same relative susceptibilities of the three species (table 2). Further tests with third-instar larvae involving 48-hour exposures and separate consideration of the dead and the moribund larvae are shown in table 3. Comparison of these data with table 1 indicated general agreement on species susceptibility but showed third-instar larvae were less susceptible than second-instar larvae.

To evaluate the possible effects of organic matter in reducing the toxic effects of metaboric acid on mosquito larvae, test preparations were made using 100 grams of humus per liter of solution and metaboric acid concentrations that produced complete mortality of third-instar larvae for each species. No reduction in the toxic effects of the metaboric acid was shown.

PUPAE.—Pupae, less than 1 day old, were held in solutions containing 1,500 to 16,000 p.p.m. metaboric acid. Results (table 4) indicate that the pupae are much less affected by the metaboric acid. In the pupal stage *Aedes aegypti* appears as the least susceptible species, and *Anopheles quadrimaculatus* remains the most susceptible species.

In addition to evaluations for 24- or 48-hour periods, the effects of prolonged exposures were made by rearing newly hatched larvae in various concentrations of metaboric acid and determining the number of adults that successfully emerged. Concentrations of 10, 25, 50, and 100 p.p.m. metaboric acid produced no significant reduction in adult production of *A. aegypti* or *C. quinquefasciatus* when compared with control larvae. At 250 p.p.m. metaboric acid, however, only 1% of the *A. aegypti* and 3% of the *C. quinquefasciatus* reached the adult stage. *A. quadrimaculatus* was again more susceptible, since 50 p.p.m. metaboric acid allowed only 2% of the larvae to reach the adult stage. At 5, 10, and 25 p.p.m. metaboric acid had no effect against the species.

Table 2.—Per cent dead and moribund third-instar larvae of *Anopheles quadrimaculatus*, *Aedes aegypti*, and *Culex quinquefasciatus* after 24-hour exposure in various metaboric acid solutions.

$B_2O_3 \cdot H_2O$ (p.p.m.)	*quadrimaculatus*	*aegypti*	*quinquefasciatus*
250	39	—	—
500	44	4	—
750	57	—	—
1,000	75	—	—
1,500	95	33	—
2,000	96	49	2
2,500	—	78	17
3,000	—	89	16
3,500	—	96	53
4,000	100	100	83
5,000	—	—	99
10,000	—	—	100

Table 3.—Per cent dead and moribund[a] third-instar *A. quadrimaculatus*, *A. aegypti*, and *C. quinquefasciatus* larvae with 24- and 48-hour exposures to various metaboric acid ($B_2O_3 \cdot H_2O$) solutions.

	quadri-maculatus		*aegypti*		*quinque-fasciatus*	
$B_2O_3 \cdot H_2O$ (p.p.m.)	24-Hr.	48-Hr.	24-Hr.	48-Hr.	24-Hr.	48-Hr.
0	0	0	0	0	0	0
100	0	0	—	—	—	—
500	0	24 (32)	0	0 (12)	—	—
1,000	—	—	—	—	0	8 (24)
1,500	—	—	0	4 (40)	—	—
2,000	—	—	—	—	0	20 (40)
2,500	44	100	—	—	—	—
3,000	—	—	8 (76)	88 (12)	—	—
4,000	—	—	—	—	32	52 (48)

[a] Figures in parentheses indicate percentage moribund larvae.

Table 4.—Percentage of pupae of *Anopheles quadrimaculatus*, *Aedes aegypti*, and *Culex quinquefasciatus* that failed to produce adults when held in various metaboric acid solutions.

$B_2O_3 \cdot H_2O$ (p.p.m.)	*quadrimaculatus*	*aegypti*	*quinquefasciatus*
1,500	36	16	32
2,000	44	32	60
3,000	64	75	76
4,000	77	48	88
6,000	100	61	96
8,000	100	76	88
12,000	100	96	100
16,000	100	100	100

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Ronnel Sprays for Systemic Control of Cattle Grubs[1]

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In addition to using ronnel as oral or subcutaneous treatments for the systemic control of cattle grubs, Roth & Eddy (1957) applied sprays onto the backs of cattle visibly infested with grubs. They found that 2.5% sprays gave complete kill, but control was 50% or less at 1% concentrations. Brundrett *et al.* (1957) found that cattle sprayed with a 1% emulsion or suspension before the grubs emerged had fewer grubs than untreated animals. The significance of the reduction was questionable. Only partial (36%) control of grubs was afforded by complete cover of 1 gallon of 0.75% or top-line spray of 2 quarts of a 1.5% spray (Howell *et al.* 1958).

Sprays containing 2.5% of ronnel can be applied to cattle

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without poisoning (Radeleff, unpublished). Because lower concentrations were partially effective and such a high concentration had not been used as a systemic treatment for grubs, a small number of cattle were treated at the Kerrville, Texas, laboratory with 2.5% sprays. The results of this test and subsequent tests with single or multiple treatments with lower concentrations are reported in this paper.

SINGLE TREATMENT.—In the fall of 1957 Hereford calves were purchased from a ranch in South Dakota. Cattle from this area had been used in previous tests (McGregor & Bushland 1957, Graham 1958) and were uniformly and heavily infested with both the common cattle grub (*Hypoderma lineatum* (DeVill.)) and the northern cattle grub (*H. bovis* (L.)).

On November 13 four animals were sprayed with a 2.5% suspension of the purified grade (Trolene) of ronnel from a 25% wettable powder at a dosage of 1½ gallons per animal with a power sprayer operating at a pressure of approximately 100 pounds per square inch. The cattle had a winter coat of long, heavy hair, and the operator held the nozzle about 1 foot from the animals to drive the insecticide onto the skin.

The treated and 20 untreated cattle were examined for grubs five times from January through April 1958. As grubs encysted they were located on maps of the back of each animal, and the total number for each animal noted. No grubs appeared in the backs of the cattle treated with ronnel. All the untreated cattle were infested and had an average of 36.3 grubs per animal.

On May 16 a group of 29 two-year-old Hereford heifers on a ranch near Mountain Home, Texas, was sprayed with a 1.75% suspension of technical ronnel (Korlan) from a 25% wettable powder at a dosage of 1 gallon per animal with a power sprayer operating at a pressure of 250 pounds per square inch. The animals were in short summer hair, and appeared to be thoroughly wetted with the spray.

The 29 treated and 38 untreated cattle were checked twice during the period of grub emergence. At the first examination (October 8) only a few cattle were infested; treated animals had an average of 2.2 and untreated animals only 1.6 grubs. At the second examination (December 17) all treated and untreated cattle were infested; treated cattle averaged 4.0 and untreated 10.7 grubs per animal. Thus, the treatment was only partially effective (63%).

MULTIPLE TREATMENT.—Because these single treatments afforded some measure of grub control, in 1958 a test was conducted to determine the effectiveness of the same total amount of insecticide applied at lower concentrations at several intervals.

A herd of Hereford cattle was divided into four groups—three groups sprayed with ronnel (Korlan), one each at 0.25%, 0.5%, and 0.75%, and the fourth with toxaphene at 0.5%. Treatments were started on May 6, and all animals were resprayed whenever necessary for control of the horn fly (*Siphona irritans* (L.)) or the lone star tick (*Ambylomma americanum* (L.)). Suspensions were applied on May 6 and 27 and July 24, and an emulsion on June 25. Each animal received 1 gallon of spray applied at a pressure of 250 pounds per square inch.

The cattle were examined for grubs five times at monthly intervals from September through January. No grubs were found in September and only a few in October. Cumulative counts were made of grubs in each animal. As shown in table 1, no grubs appeared in the backs of animals treated four times with 0.75%. One or more of the animals in each of the other groups was free of grubs, but there was little difference in average numbers of grubs per animal.

The large difference between the 0.5% and 0.75% treatments is difficult to explain because of the small number of cattle per treatment. Further studies are necessary to substantiate the validity of the 100% control observed for the 0.75% treatment, and they may show less striking differences between the two concentrations. Spacing and numbers of treatments should be correlated with the concentrations in order to obtain control of grubs in a program for control of other livestock pests.

Table 1.—Cattle grubs in backs of cattle after four applications of ronnel sprays, 1958.

CONCENTRATION	NUMBER OF CATTLE		NUMBER OF GRUBS PER ANIMAL	PER CENT CONTROL
	Treated	Infested		
0.75	15	0	0	100
.5	15	13	6.8	7
.25	8	7	6.3	14
Untreated	14	10	7.3	—

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Improved Holders for Spray Deposit Assessment Cards[1]

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Dyed cards are commonly used to assess the distribution and atomization of aerial sprays. The usefulness of these cards in the field is often destroyed by the accumulation of standing water in bent cards, curling or fading of cards exposed to ground moisture, animal damage, and other factors.

Several years ago a wire holder was developed to hold spray cards above the ground to prevent some of the above damage. This holder was difficult to construct and did not hold the spray cards firmly. Therefore, it was not adopted for field use.

This paper describes how to make an improved card holder. It is more efficient in design and is much easier to construct than the former holder. This holder has proven practical and effective under a wide range of conditions on actual spray projects. About 150 card holders can be made per man hour in the field without elaborate equipment.

STEPS FOR MAKING WIRE CARD HOLDERS:

1. Cut off the heads of 3 nails. Drive them firmly into a plank (or stump) approximately 3 inches apart forming an equilateral triangle.
2. Cut 12-gauge galvanized wire in 40-inch pieces.
3. Make a loop on the end of each wire with pliers.
4. Attach this loop to one nail and bend the wire around the outside of all three nails making two complete turns (Fig. 1, A & B).
5. Bend the remaining straight piece of wire perpendicular to the triangles formed in step 4.
6. Remove the card holder from the nails. Superimpose the bottom triangle on the top one and twist the two triangles

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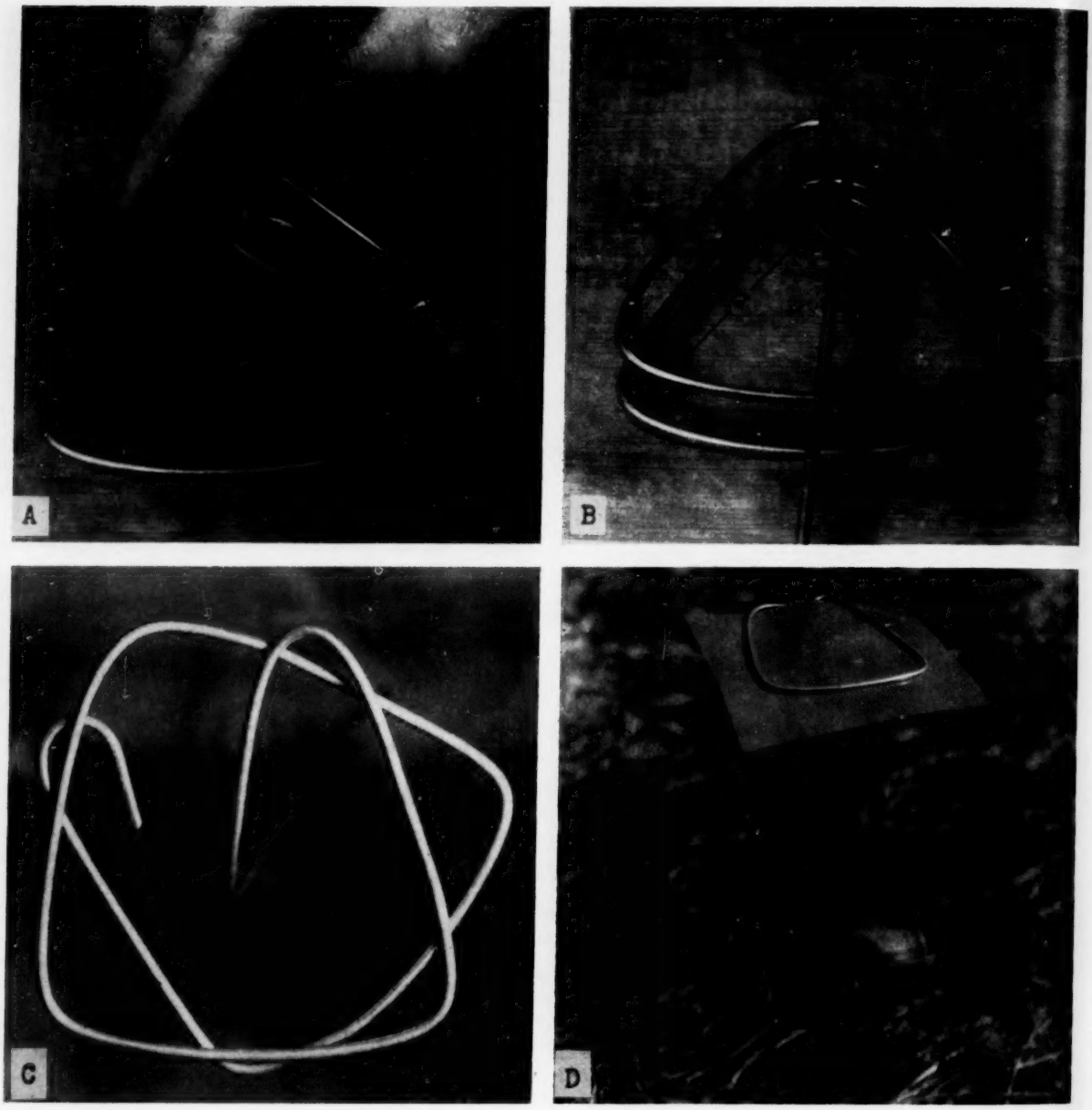

FIG. 1.—Wire holder for spray assessment card. (A, B, C) steps for making, and (D) holder in use. (Photo by H. W. White.)

in opposite directions until they form a star. This will give the holder the tension needed to hold the inserted spray card.

7. Bend the center wire (Fig. 1, C & D) into the triangles to raise the middle of the spray card, thus preventing the accumulation of standing water on the card.

Field trials have indicated that the expense of making card holders is more than offset by the following advantages: (a) no accumulation of standing water on the cards; (b) no curling and less fading of the cards owing to ground moisture and dew; (c) ease with which cards can be spotted and recovered in the field; and (d) decreased animal damage.

Wire holders can be inserted into the trunk or branches of a tree to assess the penetration of spray through the tree crown. They also can be used to sample spray drift into lakes and ponds by attaching the bases of the wire holders to anchored wooden floats.

Musca autumnalis in the Central States[1]

CURTIS W. SABROSKY, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

A second species of *Musca*, *M. autumnalis* DeGeer, has been found at a number of places in the Northeast since its discovery in 1952, including Nova Scotia, Prince Edward Island, New Brunswick, Quebec, Ontario, Maine, and New York (Long Island, Ithaca, Oswego County) (Vockeroth 1953, Sabrosky

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1957, 1959). Recently it was reported from two localities near Leesburg in northern Virginia (Sabrosky 1959), where it occurred in houses in December and January in company with cluster flies and box elder bugs.

A further jump in known distribution can now be recorded. Specimens of *M. autumnalis* were taken on June 18, 1959 at Apple Creek, Wayne County, Ohio (C. R. Neiswander), where there was said to be a severe infestation on cattle in the field. Later I received specimens collected even earlier, on June 3, 1959, in Randolph County, Indiana (D. L. Matthew), submitted by Leland Chandler, Purdue University, with the report that the flies were "always found around the eyes of cattle in pasture." The adults of *autumnalis*, whose larvae breed in cow dung, suck exudates of mammals. Vockeroth (1953) has given a key to distinguish *autumnalis* from *domestica*.

In addition to the possibility that some complaints of house flies at unusual times in late fall and winter can now be expected in the central states, attention may be called to the fact that *autumnalis* is known to be an intermediate host for *Thelazia rhodesii*, a mammalian eye worm that is prevalent in the USSR and sometimes infects a high proportion of the cattle in some areas. An American species, *T. californiensis*, is at present limited to the West Coast, and is known from sheep, dogs, and some wild animals but apparently not from cattle. It is an eye irritant and might cause blindness. If *autumnalis* spreads across the country, as seems probable, one may speculate whether it will establish a relationship to the Nearctic *Thelazia* and assist in the spread of the latter, as in the potato-*Leptinotarsa* relationship.

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Residue Studies in Sheep and Goats Dipped in 0.025% Lindane[1]

J. B. JACKSON,[2] M. C. IVEY,[3] R. H. ROBERTS[4] and R. D. RADELEFF[2] *Agric. Res. Serv., U.S.D.A., Kerrville, Texas*

The use of insecticides in the production of plant and animal foodstuffs has a great significance to public health because of the resultant residues found in these products. Controls are placed on the use of insecticides and enforced by the Food and Drug Administration. Data gathered on each insecticide is used to arrive at specified methods of proper use so that there will be no hazard to the consuming public.

MATERIALS AND METHODS.—In order to supplement previous data (unpublished) obtained at the Kerrville Station, for lindane on dairy and beef cattle, a test was conducted using sheep and goats. Eight shorn yearling Delaine wethers and 8 shorn yearling Angora muttons, in good range condition and in good health, were divided into 2 groups of 4 sheep and 4 goats each. The groups were maintained in separate pens throughout the test.

The animals of the first group served as controls and were kept free of any insecticide. The second group of animals was dipped in 0.025% lindane formulated from a 25% wettable powder. Lindane at a concentration of 0.025% is recommended by the U. S. Department of Agriculture for dipping sheep and goats for lice, ticks, and the sheep ked.

All 16 animals were biopsied (Radeleff 1950) prior to the dipping to obtain control omental fat samples for chemical analysis. After a 1-week period to facilitate the healing of operatory wounds, the second group of animals was dipped and allowed to drip dry. A schedule was established whereby two sheep and two goats from each group were biopsied on alternate 2-week periods for 12 weeks. In this manner each animal received a control biopsy and three posttreatment biopsies.

PROCEDURE FOR ANALYSIS OF SHEEP AND GOAT OMENTAL FAT SAMPLES.—The Schechter-Hornstein colorimetric method for determination of benzene hexachloride (Schechter & Hornstein) with modifications was the analytical procedure used. The following is the modification:

Weigh a 10-gram sample of fat into a Waring Blendor and blend for 2 minutes with 150 ml. of distilled chloroform. Add 15 grams anhydrous sodium sulfate and blend for 3 minutes longer. Add 1 gram of Filter-Cel, and blend for 1 minute. Filter through folded filter paper into 500 ml. Erlenmeyer flask. Wash the blender, beaker, and filter paper with about 125 ml. of chloroform.

Distill off the chloroform to approximately 200 ml.; then transfer to a 500-ml. separatory funnel. Add 0.8 ml. of a 1:1 mixture of sulfuric acid-fuming sulfuric acid for each gram of fat sample. Shake gently and let stand for the sulfonated fatty layer to separate to the top. Drain the chloroform into another 500-ml. separatory funnel. Wash the fatty layer twice with 35 ml. of chloroform and add to the other chloroform extract. Discard the funnel containing the fatty layer. Extract the chloroform three times with 35 ml. portions of the sulfuric acid mixtures. After the last extraction, allow the acid to drain down and then separate as cleanly as possible. Pour the chloroform from the top of the separatory funnel into a clean 500-ml. separatory funnel. Add 50 ml. of 5% sodium bicarbonate to the separatory funnel, stopper, invert and swirl several times with the stopcock open. Close the stopcock and shake vigorously, releasing the pressure frequently. Let stand for 10 minutes for a reasonably clear separation and then filter the chloroform layer through a 5-cm. plug of cotton packed in a glass crucible holder, into a 500-ml. Erlenmeyer flask. Wash the bicarbonate layer with 30 ml. of chloroform and filter into the Erlenmeyer flask.

Distill off the chloroform through a Synder column to a volume of 10 ml. Transfer the residue to a 125-ml. Erlenmeyer flask using 50 ml. of acetic acid to make the transfer. Add a glass bead and attach a Snyder column to the flask. Suspend a thermometer into the Snyder column so that the bulb is just below the top of the column. Evaporate the solvent until the temperature reaches

[1] Accepted for publication May 25, 1959.

[2] Veterinarian, Animal Disease and Parasite Research Division.

[3] Chemist, Pesticide Chemicals Research Branch, Entomology Research Division.

[4] Entomologist, Entomology Research Division.

Table 1.—Parts per million of lindane in the fat of sheep and goats dipped in 0.025% concentration of the Insecticide.

ANIMAL NUMBER	P.P.M. LINDANE IN FAT (WEEKS AFTER TREATMENT)					
	2	4	6	8	10	12
Goats						
4682	3.20		0.15		0.14	
4684		1.56		0.15		0
4686		1.15		.22		—[a]
4687	2.14		.40		.23	
Sheep						
4688	4.30		.37		.27	
4692		1.50		.12		0
4694		2.00		.40		0
4695	4.15		.81		.27	

[a] Fat sample not available.

the boiling point of acetic acid. Remove the Snyder column and let cool to room temperature.

Add 3 grams of malonic acid and 4 grams of 40 mesh zinc to the flask. Attach it to the Schechter-Hornstein still and follow the method as described.

RESULTS.—The residues of lindane in fat obtained from this test are given in table 1. At each biopsy period a slightly higher residue was found in sheep than in goats. The residue was highest at 2 weeks after dipping; the sheep showing an average of 4.22 p.p.m. and the goats an average of 2.67 p.p.m. It decreased steadily until at 12 weeks after dipping the residue was zero.

No symptoms of abnormal conditions were observed in the test animals.

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Changes in Common Names of Insecticides

CARROLL N. SMITH, *Chairman, Committee on Insecticide Terminology*

A revised list of common names of insecticides approved for use in the publications of the Entomological Society of America was published in the April 1959 issue of the JOURNAL OF ECONOMIC ENTOMOLOGY (52: 361–2). The changes listed below have been approved by the Committee on Insecticide Terminology of the Entomological Society of America. The listing of D-D mixture as a common name was in error, since D-D is a registered trademark and not available for use as a common name. The new name added to the list designates a compound that is an attractant for the Mediterranean fruit fly, *Ceratitis capitata* (Wied.).

Delete:

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS
D-D mixture	mixture of 1,2-dichloropropane and 1,3-dichloropropene	D-D, Dowfume N

Add:

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS
chlorbenside	*p*-chlorobenzyl *p*-chlorophenyl sulfide	Mitox
medlure	*sec*-butyl 4 (or 5)-chloro-2-methylcyclohexanecarboxylate	ENT-30992
phorate	*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate	Thimet, Am. Cyanamid 3911

OBITUARIES

Clair Cahill Alexander
1911-1959

The sudden and tragic passing of C. C. Alexander in a commercial airlines disaster over Chase, Maryland, on May 12, 1959, came as a deep and unbelievable shock to his many friends and associates in the entomological profession and in the chemical industry. His warm and friendly personality as well as his high professional ability and integrity left an indelible mark on all those who were associated or acquainted with him. It is doubly tragic that such a man should be taken during the peak of his career.

He was born on December 3, 1912, in Dayton, Ohio, and following a residence of some years in Lykens, Ohio, where he attended grade school, the family returned to Dayton. He graduated from Dayton's Roosevelt High School in 1930.

"Alex," as he was known to his many friends and associates, majored in Entomology at Purdue University where he received the Bachelor of Science degree in Agriculture in 1938. Following graduation from Purdue, he worked as an Entomological Aid at the Vincennes, Indiana, Field Laboratory of the U. S. Department of Agriculture where he developed a lifetime interest in apple insects and their control.

He undertook graduate studies in Entomology at the Ohio State University and was awarded the Master of Science degree in the spring of 1940. Alex immediately thereafter accepted a position of Entomologist with the U. S. Department of Agriculture Field Research Station in Yakima, Washington. The work in Yakima consisted primarily of research on the biology and control of apple insects with special emphasis on the codling moth. He remained with the U.S.D.A. in Yakima until late 1944 and during this period he was author or coauthor of several publications relating primarily to codling moth biology and control. His studies on mechanical traps versus bait traps for measuring moth flight and population were of special interest to workers in this field.

In late 1944, Alex accepted a position of Research Fellow on the staff of the Crop Protection Institute, Division of the National Research Council, at the University of New Hampshire. Here he pioneered in the early research on DDT insecticides under a wartime Fellowship sponsored by the Geigy Chemical Corporation. He spent the summer of 1945 on a field research project in Winchester, Virginia, evaluating DDT insecticides for apple insect control as a part of the Fellowship program.

In late 1945, Geigy decided to open their own research laboratories and he joined Geigy as Research Entomologist on October 1, 1945. He was instrumental in the design, construction, organization and operation of a complete laboratory in Bayonne, New Jersey, for chemical and biological pesticide research and screening of new compounds.

In 1951, he became Chief Entomologist, and in late 1953, he became Research Manager of Geigy Agricultural Chemicals, Division of Geigy Chemical Corporation, which position he held at the time of his death.

During this period Alex became one of the foremost experts in the chemical industry in the field of development of new pesticides. He was an active participant in the 1950 Tolerance Hearings held by the Food and Drug Administration preparatory to the establishment of pesticide residue tolerances. He became an expert in the handling of new pesticides under the requirements of the "Miller Bill" amendment to the Food and Drug Law following its passage a few years later. He was an invitation speaker on numerous occasions on one phase or another of the field of new pesticide development. He was active as a member of several technical committees of the National Agricultural Chemicals Association and was Chairman of its DDT Technical Committee.

Clair Cahill Alexander

He joined the former American Association of Economic Entomologists in 1939 and was a member of numerous Branch and Entomological Society of America (ESA) committees. He was Chairman of the Eastern Branch in 1957, and he was currently a member of the ESA Special Committee on Constitutional Amendments. He was always active in Branch and National Society affairs and he was widely consulted on such matters.

On June 22, 1940, he married Miss Isabelle Mounts of Grove City, Ohio, in Yakima, Washington. In addition to his wife, he is survived by two children, Dianne, 13, and Patricia, 8, residing at 29 Warren Avenue, Tarrytown, New York; by his father, Mr. J. L. Alexander in Dayton, Ohio; a brother, M. E. Alexander, in Silver Spring, Maryland; and a sister, Mrs. Ruth Alexander Johnson, in Dayton, Ohio.

With the help and suggestions of his many friends, a C. C. Alexander Memorial Scholarship Fund has been established at Purdue University for deserving students in Entomology. This is a perpetual fund with the income available each year for Scholarship awards, and it is administered by the Purdue Alumni Scholarship Foundation. Awards will be made in accordance with provisions stipulated by the Department of Entomology and the Purdue Scholarship Committee. This is a fitting and lasting tribute from the many who esteemed him as a colleague and friend.

GEORGE R. FERGUSON

Francis B. Foley
1906-1959

Francis B. Foley of the Whittier, Calif. station of the Entomology Research Division fell on May 2, 1959, while climbing with another man near the top of Boundary Peak, the highest mountain in Nevada. They were overtaken by a sudden snow storm or blizzard, and their bodies were not discovered until May 6.

Mr. Foley was born December 27, 1906 in Los Angeles, Calif. He grew up and attended school in Glendale, Calif., and received his degree in forestry and entomology in 1935 from Oregon State College. At various times from 1928 to 1948 he was employed by the U. S. Forest Service, the National Park Service, and the Bureau of Entomology and Plant Quarantine. He served in the Army from 1941 to 1945 and saw active duty in the South Pacific. He returned to entomological research work in 1948 and continued until his untimely death. Most of his research work was done on wireworms and bean insects. He assisted in the experiments which led to the successful control of wireworms with soil fumigants, with DDT, and with other hydrocarbons. He was the author or coauthor of numerous papers on the control of wireworms and other bean insects.

He was a lover of the outdoors, and spent his vacations and many weekends camping or hiking on the desert or in the mountains. He was much interested in boys, and on these trips frequently took several of them along with him. This was so much appreciated by these boys that a group of them requested permission to act as his pallbearers.

He was a careful and tireless worker, carrying out difficult and tedious assignments willingly and cheerfully and he was at all times an agreeable companion. His loss is keenly felt by all of his friends and associates. Mr. Foley is survived by his father, Barnett Foley, and his brother Wilbur.

ROY E. CAMPBELL

Francis B. Foley

BOOK REVIEWS

HANDBOOK OF TOXICOLOGY. VOLUME III—INSECTICIDES, COMPENDIUM by WILLIAM O. NEGHERBON. Prepared under the direction of the Committee on the Handbook of Biological Data. Division of Biology and Agriculture. The National Academy of Sciences; The National Research Council. Published by W. B. Saunders Company, Philadelphia 1959. 854+xxv pages. Price $14.00.

This massive compilation of data emphasizes the toxic effects of insecticides but it includes much more. The treatment differs somewhat from one chemical to another but that for aldrin will serve as an example. After the common name, the full chemical name and several trade names are listed. Then follow a General section indicating the uses and importance of the material; Physical and Chemical properties and a Toxicological section which is divided into: general, precautions, acute symptoms, chronic toxicity, acute toxicity to higher animals, toxicity on feeding experiments, sub-chronic and chronic toxicity, toxicity to wild birds, pharmacological, pharmacodynamic and physiological properties, phytotoxicity, hazard to wild life, toxicity to insects, toxicity compared with other chemicals, mode of action and other effects on insects; fate in the animal body. Two sections on field experience in control of destructive insects and effectiveness data from screening tests complete the nine page account.

Quantitative data are cited under most sections with reference to the source in each case. Some chemicals receive only a one or two page account containing data under only a few of the above subdivisions. Other accounts are much longer, *e.g.*, DDT 38 pages, chlordane 13 pages, organic phosphates 34 pages, fumigants 19 pages. There are 188 sections in all, of which 164 are devoted to specific insecticides and 24 are on more general topics such as fumigants, organic phosphates, bees and insecticides, acaricides, synergists, etc. There is a certain amount of duplication as in the section on organic phosphates and in the separate accounts of parathion, malathion, TEPP, etc., but this is kept to a minimum.

A 14-page introduction explains the viewpoint and the arrangement, gives definitions of many necessary terms, describes methods of testing and discusses factors affecting an insect's response to a chemical. It is an excellent account of insecticides from a general viewpoint. The bibliography of 3,404 entries goes back to the early years of this century and comes forward to 1956. An index covering chemicals by chemical, common and some trade names, plants, insects and animals completes the volume.

The foregoing account of the organization of this work cannot convey an adequate impression of the enormous amount of information nor of the clear and concise manner in which the quantitative data are set forth. This book represents the completion of a truly enormous task which called for devotion to detail without obscuring the objective of an overall picture of the hazardous nature of insecticides. It will be an indispensable tool for everyone working with these chemicals whether he be a student, researcher, agricultural advisor, insecticide chemist or technical salesman.

W. M. HOSKINS

THE SCIENTIFIC PRINCIPLES OF CROP PROTECTION, 4th ed. By HUBERT MARTIN. Edward Arnold (Publishers) Ltd., London. viii+359 pp. 1959. $12.50.

Since the third edition of this well known work appeared in 1940 entitled "The Scientific Principles of Plant Protection," many changes have taken place in this subject, especially in the field of chemical pest control. All branches of the latter (insecticides, fungicides, herbicides) have undergone revolutionary development. The greater share of the chemicals now used for these purposes was unknown in agricultural practices prior to World War II. A relatively few inorganic compounds formerly in use have been largely replaced by today's multitude of organic pesticides. Many of the latter have provided answers to control problems previously unsolved, although the use of some has introduced fresh difficulties.

The primary purpose of this book, according to the author, is to provide the plant pathologist and entomologist a review of physical and chemical aspects while the chemist and physicist are given a view of the biological side of crop pest control. Separate chapters are devoted to "Plant Resistance," "The Influence of External Factors on the Susceptibility and Liability of the Plant to Attack," "Biological Control," and "The Treatment of the Centres and Vectors of Infection." Eleven chapters are devoted to the various aspects of chemical pest control. These include chemical weed control, fumigation, seed treatment, soil treatment, and three chapters on (1) inorganic insecticides, (2) naturally-occurring contact insecticides, and (3) synthetic contact insecticides. Structural formulas are shown for a large proportion of the organic compounds. A chapter on "The Measurement and Mechanics of Toxicity" includes principles of bioassay and hypotheses concerning the mechanics of toxic action.

The text is profusely supplied with references to the literature, lists of which are located at the end of each main division of a chapter. A two-column author index occupies 16 pages, a detailed subject index 33 pages.

One notes that, while rotenone extracts are discussed, the author fails apparently to state that rotenone insecticides, at least in the United States, are largely dust formulations of ground cube root. The extensive use of ethylene dibromide as a soil fumigant is not indicated. The reference to the recent program of Mediterranean fruit fly eradication in Florida is confined to the traps used in checking on the success of insecticide applications. Eradication was actually by means of the attractive protein hydrolysate spray poisoned with malathion. Inorganic weed killers and some of the space fumigants receive little or no consideration presumably because they are not employed in the protection of growing crops.

Many would be discouraged from the task of revising such a book by the rapid development of new chemicals and the resulting control problems. Dr. Martin is to be commended for successfully completing this revision of his valuable book. It will be a welcome addition to many an entomologist's book shelf.

HAROLD H. SHEPARD
U. S. Department of Agriculture

MITES, OR THE ACARI, by T. E. HUGHES. University of London, the Athlone Press; and Essential Books Division, Oxford University Press, 16-00 Pollitt Drive, Fair Lawn, New Jersey. 225 pp. July, 1959. Price $6.75.

When the present impetus for the study of mites was inaugurated during the last World War, there was a great demand in the United States for copies of Banks' "The Acarina or Mites" published by the U. S. Department of Agriculture in 1915. "Mites, or the Acari" by T. E. Hughes is the first general treatise on mites that has been published in English since that time, and it should be even more in demand than the one by Banks

The first six chapters of this book are devoted to biological considerations of mites. Taxonomic groups are discussed in relation to the ecological distribution of various members, their feeding habits, and their importance as predators, animal parasites, and plant feeders. The information presented is drawn from scattered literature, but it is not always carefully evaluated as to probable accuracy. For instance, the plant-feeding *Pseudoleptus arechavaletae* Bruyant is cited as attacking the bush turkey in Uruguay even though this observation is highly questionable.

One chapter is devoted to the subject of mites as vectors. However, no mention is made of the experimental transmission of certain diseases such as endemic typhus and tularemia by *Ornithonyssus bacoti* (Hirst). The importance of mites as vectors of plant diseases is not mentioned, even though studies were conducted in England implicating an eriophyid in the transmission of current reversion, and more recent studies have shown related mites to be vectors of fig mosaic, peach mosaic, and wheat streak mosaic.

The most important and larger portion of "Mites, or the Acari" is a survey of our knowledge of the morphology and internal anatomy of mites, and it is in the eight chapters dealing with this subject that Dr. Hughes has made personal contributions. A classification of mites made on a basis of external morphology alone is fraught with difficulties, and a comparative study of the development and functions of internal organs will aid immensely in understanding the evolution and relationships within the Acarina. This portion of the book should be an invaluable stimulus to further research along this line.

The last chapter is a listing of the family classification of mites, prepared several years ago with the aid of the eminent acarologists at the British Museum (Natural History). This subject is rapidly undergoing revision at the present time.

Excellent illustrations have been chosen to depict various types of mites and their anatomy. The use of the initials of authors following scientific names, such as "C. Linnaeus," may be to some a reading distraction; moreover it takes up valuable space in very terse writing and is not used consistently throughout the text. The use of vernacular designations such as "trombids" (for trombidiids or trombidioids) is not in good taste, and the rather indiscriminate use of "mites" for "ticks" may be confusing to some. But such criticism is trivial.

"Mites, or the Acari" is a welcome addition to the libraries of biologists and acarologists. It is gratifying to find a new scientific book in English that is reasonable in price, having been partially subsidized.

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